SELECTED APPLICATIONS OF ANALYTICAL SPECTROSCOPY TO FOOD AND ENVIRONMENTAL CHEMISTRY

by

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Dedicated to my parents.

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Chapter 1

General Introduction to the Thesis

Introduction

Since the advent of the industrial age, the environment around us has been changing more rapidly than ever before. In order to understand the implications of this changing environment on various organisms, efforts have been mounted to determine the composition of the environment. In this context, the field of environmental analysis has received a lot of attention. The reason why food analysis has become increasingly interesting to scientists is that the ingestion of food is one of the primary modes by which organisms interact with the environment.

Elemental determination is an important area of work in environmental and food analysis. As more and more information becomes available on the role of major, minor and trace elements in the various metabolic processes, it becomes increasingly necessary to develop methods to determine elemental concentrations reliably and rapidly. Some of the methods that are currently available include gravimetry, titrimetry, spectroscopic methods, electrochemical methods, mass spectrometry and neutron activation analysis. Many of these methods can be performed quite rapidly if the sample is available in the form of a solution. However, most procedures by which the samples can be solubilized are lengthy and labor

intensive. Hence, methods that can tolerate very little or no sample preparation will be extremely valuable.

The main focus of this thesis project is to apply analytical spectroscopy innovatively so that sample preparation can be minimized for elemental determinations in environmental and food samples. Chapter 2 details improvements on a near-direct slurry method for the determination of calcium by flame emission. A slurry method to determine selenium in beef liver by graphite furnace atomic absorption is described in chapter 3. In chapter 4, the diphenyl carbazide method for Cr(VI) has been shown to be applied successfully in a lignosulfonate matrix without a need for extraction procedures. In addition to this, an attempt has also been made to investigate the redox chemistry of chromium(III) lignosulfonate in the presence of chlorinated tap water.

Calcium is fairly widespread in our environment and is regarded as being quite innocuous from a toxicological standpoint. In fact, calcium is well recognized as an essential nutrient. One of the very few safety regulations placed on it is with regard to the long term exposure limits placed on certain calcium compounds. This is due to the fact that prolonged inhalation of calcium dusts and asbestos can have deleterious effects on lungs

in humans[1]. In any case, calcium is one of the most widely determined elements because of its ubiquity.

Chapter 2 deals with the specific case of the determination of calcium in mechanically deboned meats. The purpose of this determination is to get a handle on the percentage of residual bone fragments present in the meat. Determinations such as these are performed by regulatory agencies such as the United States Department of Agriculture. Such measures are necessary because modern food processing plants use deboning machines that treat the meat harshly and can introduce bone chips in meats. While the bone chips add to the weight of the meat, they add to the cost of the meat product disproportionately.

The United States Department of Agriculture performs scores of calcium determinations in mechanically deboned meats daily by lengthy wet ashing followed by EDTA titration. In addition to the long and cost inefficient wet ashing procedure, the EDTA titration is also prone to interferences due to divalent ions of zinc, magnesium and heavy metals. The flame emission method is free of the above mentioned interferences. Besides, the new method detailed in chapter 2 reduces individual sample preparation to less than five minutes through shaking of an entire batch of samples with 2% nitric acid.

Selenium is an essential trace element for many organisms including human beings and is found as selenocysteine in the enzyme glutathione peroxidase[2]. It is believed to have a role in the heme metabolism as well. Selenium deficiency in many animals may cause degenerative changes of several organs, growth retardation and failure to reproduce[1]. In humans, selenium toxicity has been suggested to lead to mutagenic and carcinogenic effects. Selenium is usually present at the trace or ultra-trace levels in most samples and is most commonly determined by hydride generation atomic absorption spectrometry. It is an extremely sensitive method and is very useful for selenium determinations in samples in solution and for the determination of the low natural levels in water samples. However, this method is also faced with the same limitation of being able to tolerate samples only in the liquid phase. Selenium can also be determined by fluorometry, atomic fluorescence spectrometry, voltammetry, isotopic dilution mass spectrometry and gas chromatography. Other methods that may not need sample dissolution such as x-ray fluorescence and neutron activation analysis are often not very accurate or cost effective. In the slurry method detailed in chapter 3, beef liver is simply

homogenized for 2 minutes with water and determined by graphite furnace atomic absorption following dilution. Although this method cannot replace hydride generation atomic absorption for liquid samples, the two techniques are complementary in terms of the sample phase.

Investigation of the redox chemistry of chromium(III) lignosulfonate in the presence of chlorinated tap water is reported in chapter 4. Cr(III) as the chloride is known to be oxidized to the hexavalent Cr(VI) by chlorinated tap water. This is of environmental significance because while Cr(III) is environmentally benign, Cr(VI) is toxic. No information is available on the oxidation of Chromium(III) lignosulfonate. It is quite possible that the lignosulfonate may stabilize the Cr(III) and prevent it from being oxidized by chlorinated tap water. Experiments were conducted to investigate this and the results are reported in chapter 4.

References.

1. H.G. Seiler, H. Sigel and A. Sigel, Handbook on Toxicity of Inorganic Compounds, Marcel Dekker, inc., New York (1988).

2. NRC, Subcommittee on selenium, Committee on animal nutrition, Board of Agriculture, National Research Council, "Selenium in Nutrition," National Academy Press, Washington, D.C., 1983.

Chapter 2

Near-Direct Flame Emission Dermination of Calcium in Mechanically Deboned Meat Products.

INTRODUCTION

A high solids nebulizer for atomic absorption spectrometry based on the principles first developed by R.S. Babington was reported by Fry and Denton[1]. The main feature of this nebulizer was that the sample did not pass through any restricted orifices. As a result, a wide variety of samples with relatively high percentages of suspended solid particles could be transported into flames and plasmas without clogging the nebulizer. Mohamed and Fry[2] used an updated version of this Babington nebulizer along with a conventional air-acetylene slot burner for direct atomic spectrochemical analyses of animal tissue. Further modification of this high solids nebulizer by Wichman, Fry and Mohamed[3] resulted in a non-wettable Teflon Babington slurry nebulizer. Fietkau, Wichman and Fry[4,5] used this for near-direct determination of sodium and potassium in hotdogs and other processed meats by flame emission. This slurry nebulizer was also used for the rapid DCP atomic emission determination of residual bone fragments in mechanically separated turkey by Wichman, Fry and Hoffman[6]. The results reported in references 4 and 5 eventually led to the development of a rapid, sequential meat analyzer for sodium, potassium and calcium as reported by Fietkau[7].

The focus of the research conducted and reported in this chapter was to further refine the autoanalyzer described in reference 7 and to shorten the procedure involved in the direct flame emission determination of bone fragments (as calcium) in mechanically deboned meats. The conventional method of sample preparation for such an analysis would involve lengthy wet or dry ashing procedures. However, the main purpose of these procedures was to solubilize the sample to make it amenable to be aspirated by conventional pneumatic nebulizers which have restricted orifices through which the sample had to pass. Since the Babington nebulizer tolerated a high percentage of solids, the wet and dry ashing steps were eliminated. The meat sample was homogenized with water using a Brinkmann Polytron homogenizer to prepare an aqueous slurry of the sample. An aliquot of the homogenate was then made 2% in nitric acid and shaken for ten seconds. As reported by Wichman[10], the homogenization step reduced the residual bone fragments to a small enough size that the nitric acid in the subsequent step extracted all the calcium from the bone fragments and brought it into solution. The acidified slurry was then nebulized directly into an air-acetylene flame. However, it was found that the autoanalyzer at times gave low recoveries

for the determination of calcium in mechanically deboned meats using the slurry method as compared to the conventional wet ashing method. In order to correct this problem, a new spray chamber - burner system was designed and its characteristics evaluated. The new system consisted of the same Babington type nebulizer. However the Jarrell-ash tapered spray chamber which was used earlier[7] was replaced by a new, cylindrical, homemade Teflon spray chamber. As before, this system also had an insert placed in the spray chamber. However, in the earlier design the spray chamber insert was insecurely placed in the spray chamber with considerable uncertainty as to its final position and therefore considerable uncertainty as to the droplet size emerging in the tertiary aerosol. This was rectified in the new design by fixing the position of the spray chamber insert using a positive locking mechanism. In the new design a positive locking mechanism was also provided to fix the position of the nebulizer insert. This was done to ensure that the sample always flowed along the groove cut into the Teflon nebulizer insert and thus over the orifice through which the oxidant gas flowed. If this were not the case, problems such as depression in sensitivity and difficulty in obtaining reproducible results could occur. Furthermore, simple aqueous calibration may no longer

yield correct results because of excessive droplet sizes or the standards and the samples could skirt around the oxidant stream in different ways. The positions of both the nebulizer and spray chamber inserts were fixed to take care of this.

Another goal of this study was to investigate the possibility of reducing the time involved in the sample preparation step in the determination of residual bone fragments as calcium in mechanically deboned meats. As outlined earlier, the procedure involved the rapid homogenization of the sample followed by shaking with nitric acid. This required that each sample be prepared individually on a sequential basis. Furthermore the homogenizer was fairly noisy and somewhat tedious to clean. The above two factors are two liabilities of the slurry method which assume considerable significance in the case of the analysis of a large number of samples as is the case with the routine analysis programs of organizations such as the United States Department of Agriculture. So the possibility of designing a 'batch' (parallel multisample) method which would do away with the noisy homogenizer was investigated. The main function of the homogenization step was to rapidly break down the solid animal tissue into micron sized particles so that

the calcium from the tissue can simply be extracted by shaking the homogenate in 2% nitric acid for 10 seconds. However, in the case of mechanically deboned meats, the tissues have already been broken down considerably by the mechanical action of the deboning machine. It was hypothesised that by simply shaking the tissue in dilute nitric acid for somewhat longer periods of time, calcium might be extracted quantitatively from the tissue. Experiments were conducted to verify this by shaking a wide variety of mechanically deboned meats for different periods of time. The calcium contents of all of these shaken samples were determined using the slurry atomization flame photometer. The results were compared with those obtained by analyzing the same samples after lengthy wet ashing procedures.

Experimental

Apparatus.

Most of the apparatus, except the spray chamber-burner system were the same as those used by Fietkau[7]. A Brinkmann Polytron PT20ST homogenizer (Brinkmann Instruments Co., Westbury, NY) with a stainless steel probe was used to homogenize the meat samples. As mentioned earlier in the introduction, the use of a simple mechanical shaker in place of the homogenizer for sample preparation will be reported in the latter half of this chapter. A mechanical shaker (Precision Scientific Co., Chicago, IL) with horizontal back and forth motion was used.

A Perkin-Elmer Coleman 51 flame photometer (Perkin-Elmer, Oakbrook, IL) fitted with a 623 nm calcium interference filter (Band pass = 10 nm) was used to measure calcium emission intensities. A variable iris diaphragm (1-33 mm) was placed before the interference filter to attenuate the flame emission.

The commercial version of this instrument consisted of an oxygen supported total consumption burner-spraychamber system. Fietkau[7] replaced this with a 13 hole (1.3 mm diameter/hole) air-acetylene burner, a Jarrell-Ash tapered polyethylene spraychamber containing a teflon spraychamber insert and a Babington type Teflon

slurry nebulizer. For the work reported in this chapter, a bigger and heavier burner head with the same 13 hole air-acetylene configuration was used. A homemade cylindrical Teflon spray chamber fitted with a Teflon insert was used along with this burner. The position of the spray chamber insert was fixed by locking it on to the spray chamber using a nylon screw. The high solids Teflon Babington nebulizer was the same as the one used by Fietkau[7] with the only modification being that the nebulizer insert was locked in position using nylon set screws.

A Rainin Rabbit peristaltic pump (Rainin Instrument Co., Woburn, MA) fitted with wide-bore tubing (2.29 mm i.d.) was used along with a Technicon autosampler (Technicon Instruments, Terrytown, NY) to deliver the samples to the nebulizer. A Cole-Parmer strip chart recorder (Cole-Parmer, Chicago, IL) was used to record the data. A Commodore 64 lab computer was also used for data collection and analysis.

Figure 2.1

The teflon nebulizer-spray chamber system.

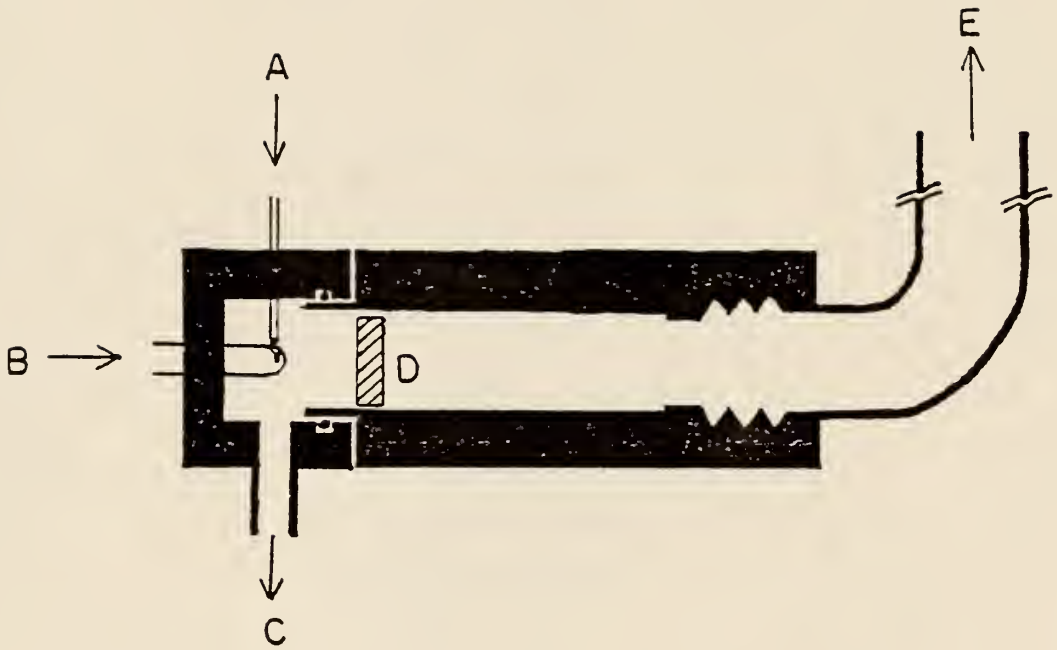
A. Sample

B. Oxidant (Compressed air)

C. Drain

D. Spray chamber insert[7,9]

E. Tertiary aerosol to the burner


A
B
C
D
E

Procedure:

Standard calcium solutions were prepared by diluting a 1000 ppm Fisher certified atomic absorption standard (Fisher Scientific Co., Fairlawn, NJ). Phosphate standards were prepared from concentrated phosphoric acid. All dilutions were performed either by volume using volumetric glassware or by weight in polypropylene bottles on a top loading balance (0.01 g readability) using distilled deionized water.

A clean 4 oz square glass bottle was zero tared on a top loading balance with 0.01 g readability. Approximately 10 g of the mechanically separated meat product was placed in it using a clean stainless steel spatula and its weight was accurately noted. Without hitting the tare bar again, distilled deionized water was added to bring the total weight to exactly 3.333 times the initial weight of the meat product (about 33.33 g). The contents were then homogenized for 2 minutes at top speed using a Brinkmann Polytron homogenizer fitted with a stainless steel probe. The homogenizer probe was then rinsed in three separate portions of distilled deionized water. The above procedure resulted in a 30 % meat slurry. About 20 g of this slurry was transferred into a clean, zero tared polypropylene bottle on a top loading balance using a clean pasteur pipet. Without hitting the

tare bar, distilled deionized water was added to this until the total weight was about 5 g below the target weight of about 120 g (exactly six times the weight of the 30 % slurry). About 2.4 g of concentrated nitric acid was added followed by careful addition of distilled deionized water to bring the total weight to the final target weight of 120 g. The polypropylene bottle was then capped and shaken for 30 seconds. The resulting slurry was 5% in solid meat tissue and 2% in nitric acid.

Alternatively, calcium in mechanically separated meat products was also extracted by employing a mechanical shaker instead of the homogenizer. A 4 oz glass bottle was zero tared on a top loading electronic balance with 0.01 g readability. About 10 g of the meat product was added to it using a clean stainless steel spatula. Without hitting the tare bar, distilled deionized water was added to bring the total weight up to about 95 g. About 2 g of concentrated nitric acid was then added followed by the careful addition of distilled deionized water to reach the target weight of about 100 g (exactly 10 times the initial weight of the meat tissue). The bottle was then capped tightly and shaken for 20 minutes in the mechanical shaker. The resulting slurry was 10% solid meat tissue and 2% nitric acid.

The meat slurries were introduced directly into the analyzer without filtering them as no clogging was encountered in pumping them through wide bore tubing.

RESULTS AND DISCUSSIONS

Phosphate Interference Studies:

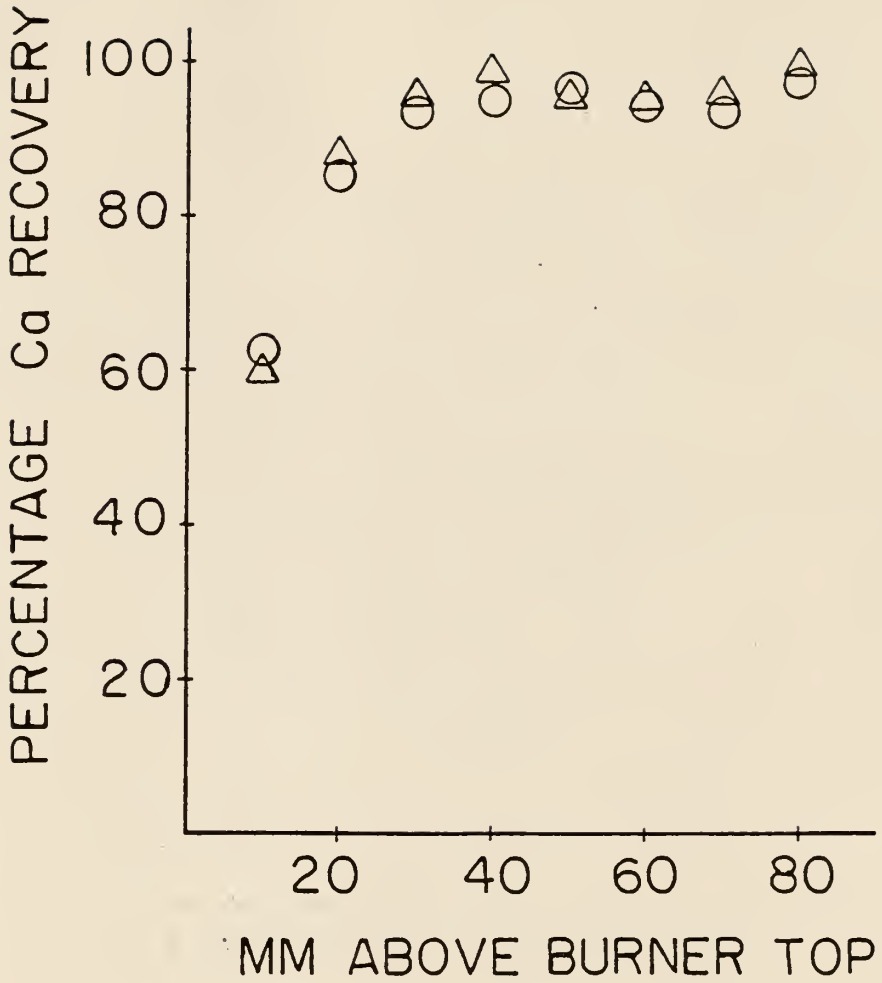
It is well known that the presence of phosphates lowers the atomic absorption and emission signals of calcium in premixed air-acetylene flames. This is due to the slower atomization rate of calcium phosphate as compared to calcium chloride or nitrate in the air-acetylene flame. In the hotter nitrous oxide-acetylene flame, atomization is markedly accelerated and reaches completion for all three salts prior to the spectral observation zone. However, due to burning velocity (i.e. safety) considerations, the air-acetylene combustion mixture was preferred for this work[7]. Smith and Browner[9] found that the phosphate interference in aqueous samples could be overcome by reducing the sizes of the tertiary aerosol droplets reaching an air-acetylene flame. They achieved this by placing inserts (baffles) within the spray chamber fitted with a capillary pneumatic nebulizer. Fietkau[7] found that the phosphate interference could be reduced for a system fitted with the Babington nebulizer by using a single spray chamber insert. In the work reported here only one spray chamber insert similar to the one used by Fietkau[7] was used in conjunction with a similar Babington nebulizer. The new spray chamber was

Figure 2.2

Percentage calcium recovery **versus** height of observation above the top of the burner head.

○ - old spray chamber - burnersystem(reference7).

△ - new spray chamber - burner system.


PERCENTAGE Ca RECOVERY
100
80
60
40
20
20
40
60
80
MM ABOVE BURNER TOP

shown in figure 2.1 and may be compared with the earlier system of Fietkau[7].

The emission intensities obtained from four calcium standards (40 ppm, 80 ppm, 150 ppm and 300 ppm) were compared with those from the same four standards with 450 ppm phosphate added to each of them. The emission intensities were measured as a function of the height of observation above the top of the new burner head. Percentage calcium recovery was calculated as follows:

$$\% \text{ Ca recovery} = \frac{I\ (\ Ca + \text{phosphate}\ )}{I\ (\ Ca\ )} \times 100$$

where I (Ca + phosphate) is the relative emission intensity of the calcium standard with phosphate added to it and I (Ca) is the relative emission intensity of the calcium standard with no phosphate added. The % Ca recovery for the four standards were then averaged and plotted against the height of observation (figure 2.2). The general shape of the calcium recovery curve is similar to that determined by Fietkau[7], but the plateau is reached faster here. From this curve it can be concluded that as long as the height of observation above the top of the burner head is at least 40 mm, phosphate interference is not encountered with the new system.

Table 2.1

Solid Phase Calcium Concentrations (%)

MDM Type	Wet ashed samples		slurry atomization flame emission	
	EDTA* Titration	Plasma@ Emission	old# system	new& system
chicken	0.080	0.082	0.072	0.100
chicken	0.090	0.099	0.089	0.110
chicken	0.120	0.057	0.048	0.057
chicken	0.170	0.170	0.145	0.160
beef	0.050	0.036	0.031	0.038
beef	0.050	0.040	0.036	0.042

Note: The four chicken samples were all different. The two beef samples were also different.

*Results obtained by USDA analysts at Midwestern laboratory, St.Louis.

@Analysis done at KSU-CESL (analyst- D.E.M.), Summer '86. Nitric acid wet ashed samples.

#Analysis done at KSU (analyst- R.F.), Spring '86. old system (reference 7).

&Analysis done at KSU (analyst- P.V.), Summer '86.

Calcium Recovery by Homogenization:

The new slurry nebulizer, burner head and spray chamber helped alleviate previous low recovery and instability problems. The new design resulted in a much stabler flame and so in much less baseline noise. As a result, an entire calibration range from 15 ppm to 250 ppm Ca could be covered in a single run, whereas the previous system had to cover this range with two separate dilutions. The new positively locked nebulizer and spray chamber inserts also helped achieve consistently good calcium recoveries as can be seen from Table 2.1.

In Table 2.1 it is worth noting that the new flame emission system produced calcium recoveries that were on the average **6% higher** than the results obtained by the high resolution direct current plasma emission method. In contrast, the old flame emission system produced results that were on the average **13% lower** than those obtained by the DCP method. Although not reported here, more meat samples were analyzed by the plasma and the new flame emission methods and it was determined that the new flame emission method produced results that were on the average **2% higher** than those obtained by the plasma method (see appendix E). This is less than the percentage relative standard deviations of the methods involved and is not deemed to be a significant difference especially when the

sample heterogeneity is considered along with the relatively small sample size employed with the wet ashing DCP method (0.5 - 2.0 g). Comparisons of this type will however be subject to sub-sampling errors inherent with small amounts of heterogeneous meat wet ashed for DCP analysis. To avoid sampling errors with food, FDA recommends a minimum of 30 g and USDA uses 10 g. The new slurry method is capable of minimizing sampling errors because a 10 to 20 fold larger sub-sample may be processed. Comparisons between the flame emission and the EDTA titration methods are difficult to make, in view of the well known fact that EDTA titrations are prone to interferences by other cations present in the samples, especially Mg^{+2} ions. Divalent cations and other transition metal ions can contribute to the endpoint of the titration. In many cases involving heavy metal cations the problem can be alleviated by using masking agents such as CN^{-}, $S^{=}$, and hydroxylammonium chloride. On the other hand, Mg^{+2} ion cannot be masked and so high calcium concentrations are often reported. Since the DCP method is virtually interference free it was the preferred 'reference method' to be used for comparison purposes.

Figure 2.3

Percentage Calcium recovery from mechanically deboned beef **versus** duration of shaking.

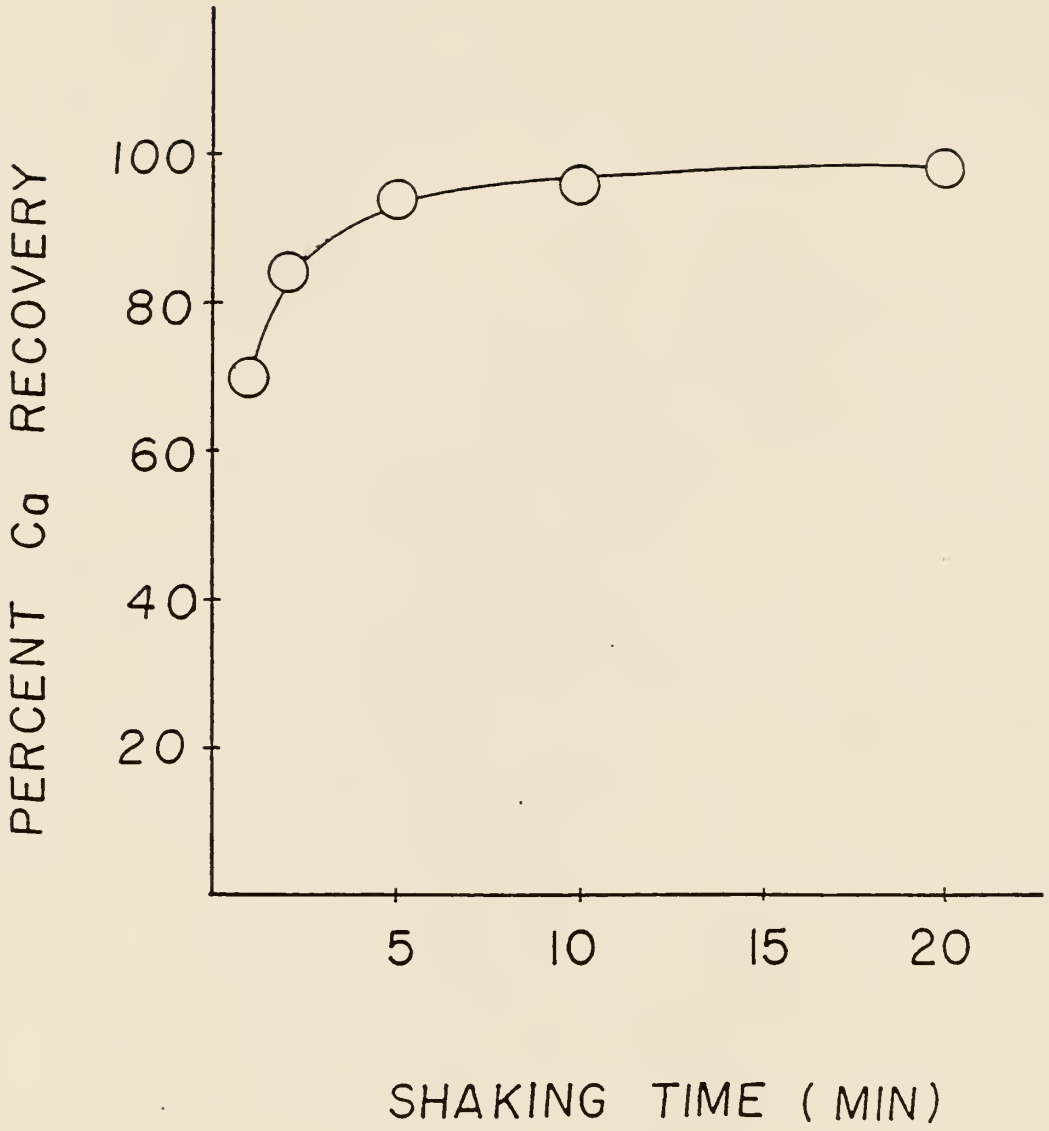

PERCENT Ca RECOVERY
100
80
60
40
20
5
10
15
20
SHAKING TIME (MIN)

Figure 2.4

Percentage Calcium recovery from mechanically deboned chicken **versus** duration of shaking.

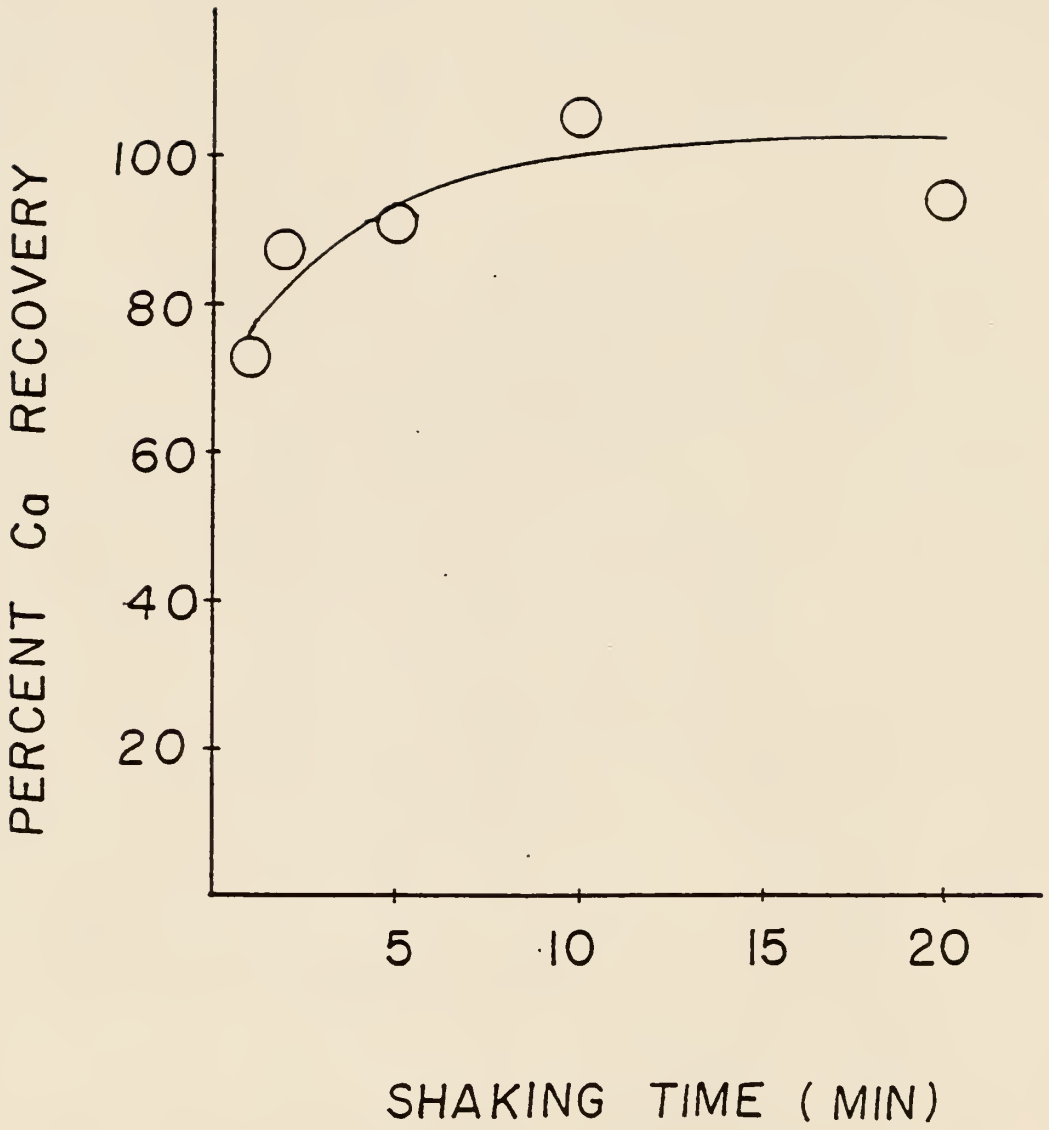
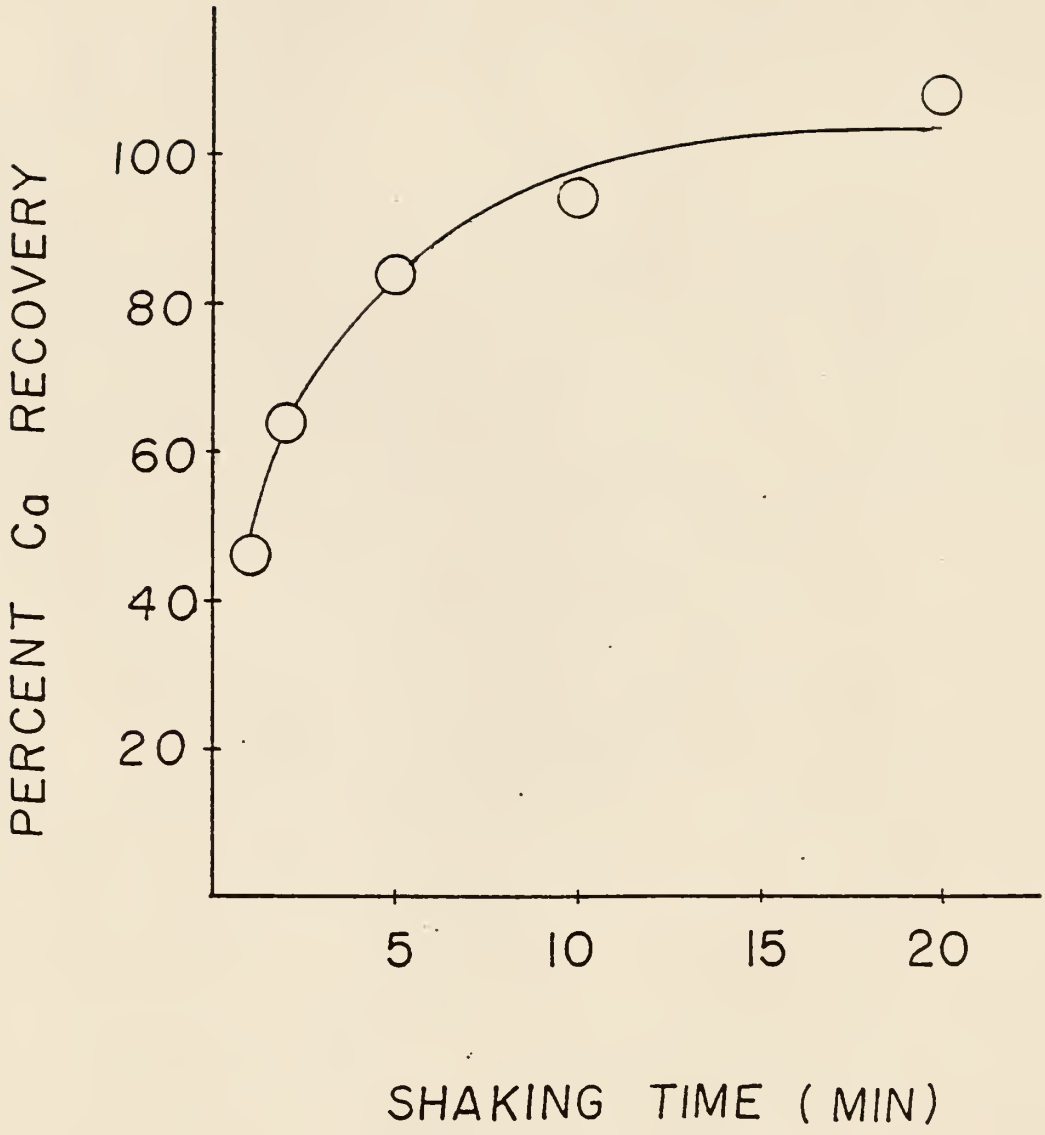

PERCENT Ca RECOVERY
100
80
60
40
20
5
10
15
20
SHAKING TIME (MIN)

Figure 2.5

Percentage Calcium recovery from mechanically deboned pork **versus** duration of shaking.


PERCENT Ca RECOVERY
100
80
60
40
20
5
10
15
20
SHAKING TIME (MIN)

Calcium recovery by shaking :

The mechanical shaker proved quite effective in extracting the calcium from a variety of mechanically deboned meat products. Figures 2.3, 2.4 and 2.5 summarize the results of the calcium recovery tests conducted with three different kinds of meats namely beef, chicken and pork. Each of these three kinds of meats was shaken with 2% nitric acid for periods up to 20 minutes and were analyzed using the new flame emission system. Calcium recovery percentages were computed based on the calcium determinations of the same samples by the direct current plasma emission method following lengthy wet ashing of the samples. It can be seen that the calcium from the meat samples was quantitatively extracted in 20 or perhaps even 10 minutes. However, it is important to recognize that the shaking time versus recovery studies may be specific to the shaker used in this work and could vary depending on the type of shaker, size of the containers and the amount of the sample used. The size of the shaker and the samples used in this work permitted us to shake up to 48 samples at one time. So once the samples were thawed and the 2% nitric acid was prepared, it took **less than 2 minutes of sample preparation time per sample.**

Table 2.2

Sample Type	% Calcium in solid phase	
	Wet ashed Sample (Plasma Emission)	Slurry Method (Flame Emission)
Chicken	0.097[@]	0.096[*]
Chicken	0.19[@]	0.17[*]
Beef	0.036[#]	0.035[@]
Beef	0.11[#]	0.11[@]
Pork	0.025[#]	0.026[@]
Turkey	0.17[@]	0.12[*]

[@] Obtained by Analyst-1 (P.V.) at KSU, fall '86.

[#] Obtained by Analyst-2 (D.E.M.) at KSU-CESL, summer '86.

[*] Obtained by USDA analysts (J.M., D.M.) at Midwestern Laboratory, St. Louis, fall '86.

In order to verify that the shaker operated consistently providing quantitative extraction of calcium from the mechanically deboned meat products, several more samples were analyzed. The results are displayed in Table 2.2. This indicated that the mechanical shaker in conjunction with the flame photometer provides for near-direct determinations of calcium in mechanically deboned meats.

Next, an attempt was made to extend the method to determine dairy additives as calcium in hotdogs. Regular hotdogs were analyzed side by side along with cheese hotdogs for comparison. The results are compared to the reference method (wet ashing followed by DCP emission) in Table 2.3. It is important to note that in this case, the hotdogs were simply cut into cubes approximately 10 mm on a side and shaken for 20 minutes in 2% nitric acid. Fairly good calcium recoveries were obtained as can been seen from the Table. In our belief, this is further indication that mechanical shaking with dilute acid is a viable method for calcium determination in meat products. Agreement of the new procedure in this chapter has generally been well within the limits of sampling error inherent with the relatively small sample sizes of

Table 2.3

Calcium in Hot dogs (dairy additive)

Sample	% Ca (original solid)	
	Wet ashed Sample	Slurry Method
Cheese Hotdog	0.11	0.094
Ca-reduced Hotdog	0.016	0.014

heterogeneous meat that can be handled by the "reference" methods.

References.

1. R.C. Fry and M.B. Denton, Anal. Chem. **49**, 1413 - 1416 (1977).

2. N. Mohamed and R.C. Fry, Anal. Chem. **53**, 450 -455 (1981).

3. M.D. Wichman, R.C. Fry and N. Mohamed, Appl. Spectrosc. **37**, 254 (1983).

4. R. Fietkau, M.D. Wichman and R.C. Fry, Appl. Spectrosc. **38**, 118 -124 (1984).

5. M.D. Wichman, R. Fietkau and R.C. Fry, Appl. Spectrosc. **40**, 233 -238 (1986).

6. M.D. Wichman, R.C. Fry and M.K. Hoffman, Appl. Spectrosc. **40**, 351 -355 (1986).

7. R. Fietkau, Ph.D. Dissertation, Kansas State University, Manhattan, Kansas (1986).

8. C.Th.J. Alkemade and M.H. Voorhius, Fresenius Z. Anal.Chem. **163**, 91 - 103 (1958).

9. D.D. Smith and R.F. Browner, Anal. Chem. **56**, 2702 - 2708 (1984).

10. M.D. Wichman, Ph.D. Dissertation, Kansas State University, Manhattan, Kansas (1984).

Chapter 3

Slurry Atomization Graphite Furnace Atomic Absorption Determination of Selenium in Beef Liver using Palladium Matrix Modification.

Introduction

Graphite furnace atomic absorption spectroscopy is one of the most widely used and important tools for elemental determination at ultra-trace levels. The earliest reported GFAAS system was built by B.V. L'vov in 1958[1]. Improvements on the original system by Massman[2] and Woodriff[3] eventually led to the first commercial instrument in 1970. Since then, many more new developments have been incorporated into commercial GFAAS instruments such as micro-processor controlled heating, pyrolytic carbon coated tubes and the platform-furnace technology. GFAAS has reached a level of automation wherein sequential multi-element analyses can be performed on a host of samples without operator intervention once the analysis has been started.

There are several advantages to the GFAAS method that make it a very attractive and viable tool for ultra-trace elemental analysis. The main ones are outlined below:

1. It is an extremely sensitive method. The detection limits for most elements are in the sub part per billion range.
2. Very small sample quantities are sufficient. Usually, no more than 20 μL of the sample is required for a single analysis.

Any quantitative determination essentially consists of two parts: sample preparation and analysis. Conventionally, most analysts need to resort to the use of elaborate and meticulous sample preparation methods for ultra-trace anlyses by GFAAS. This is due to several reasons. Firstly, graphite furnace analyses are prone to a number of interferences[4]. As a result, the analyst strives to solubilize the analyte in a clean and homogenous matrix with a hope of reducing background and other interferences as much as possible. This is achieved by time intensive wet or dry ashing procedures which are sometimes followed by extraction procedures. Secondly, since the analyte is present in very low concentrations, contamination, analyte volatilization and losses due to excessive sample transfers cannot be tolerated. Thirdly, the furnace is not very tolerant either of perchloric acid (which destroys the graphite by oxidation) or hydrochloric acid (which induces excessive molecular spectral background in the presence of alkali and alkaline earth matrix salts). As a result, sample preparation is even more of an error-prone and time consuming bottleneck in performing graphite furnace atomic absorption analyses of solid tissue than it was for flame and plasma atomization.

Many workers have striven to reduce the sample preparation time and several different approaches have been taken in this direction. Direct Solid sampling of fine, dry powders is one of the growing popular approaches because this requires very little or no sample preparation. While this works relatively well with homogeneous dry, powdered samples, whole, wet heterogenous samples such as biological tissues are often not amenable to solid sampling techniques due to problems in obtaining samples reproducibly and delivering a representative sub-sample to the furnace[11]. Another approach is to disperse the sample in a liquid medium to make a suspension or slurry of the sample. Fry and co-workers[28,29] have shown that in flames and plasmas the key to obtaining good elemental recovery and reproducibility is to reduce the particle sizes of the suspended solids for efficient sample delivery, nebulization, aerosol transport and atomization in flames and plasmas. A wide range of samples ranging from animal and plant tissues to coal and geological materials have been shown to be accurately and precisely determined using this method which has come to be known as "slurry atomization". Following up on these successful initial approaches in flame and plasma systems, Fietkau[30] extended the slurry method to graphite furnace systems.

So far methods have been developed to determine lead, cadmium, nickel and chromium in beef liver by this slurry method.

The salient features of the slurry method for graphite furnace analysis developed by Fietkau[30] are worth reviewing at this point.

1. The beef liver sample was rapidly homogenized (2 min) with water using a Brinkmann Polytron homogenizer to produce a 10% slurry.
2. This method used commercial instrumentation without any modifications. Automated sample delivery micropipetting systems with narrow bore tubing made for samples in the solution phase were used without any alteration since reproducible slurry sampling could be obtained without any clogging. The particle sizes of the solids were sufficiently small and a small amount of surfactant was added to the slurry.
3. "In furnace" oxygen ashing was employed in order to remove the organic material effectvely prior to atomization. This allowed the use of much higher ashing temperatures without premature volatilization loss and ultimately served to lower the spectral background to manageable levels.
4. Accurate results were rapidly obtained for lead,

cadmium, chromium and nickel.

The goal of the research conducted and reported in the present study was to further expand the scope of the slurry method for GFAA analysis by including selenium as one more element to be determined by this method. This element is considerably more difficult in that premature volatilization during the ash cycle is more likely and the wavelength is considerably shorter and as a result the nonspecific spectral background absorbance will be more pronounced during atomization.

Several authors have reported a number of methods for determining selenium by GFAAS in a variety of matrices[4,5-24]. However, almost all of them involve lengthy wet or dry ashing procedures. Many authors have also reported loss of selenium by volatilization during sample preparation and atomization steps due to the low boiling points of many of its oxides, chlorides and oxychlorides. The boiling points of some of the compounds of selenium that may be formed in a furnace are shown in Table 3.1. As a result, the use of a matrix modifier becomes indispensible during analysis. The function of these matrix modifiers is to stabilize the selenium by forming stable intermediate species that are not prematurely volatilized during the heating stages prior to atomization. Some of the modifiers that have been

Table 3.1

Boiling points of Selenium Compounds

	Boiling Point
Se	685 °C
SeO_2	350 °C (sublimes)
SeO_3	180 °C (decomposes)
Se_2Cl_2	130 °C (decomposes)
$SeCl_4$	196 °C (sublimes)
$SeOCl_2$	176 °C

tried are nickel[4,6,12,14,15,21,27], palladium[10,17-18,20,24], copper[9,16,22,26], magnesium[9,12,16,18,26] and to a lesser extent, silver[4,7]. Of these, nickel and palladium are most commonly used. In this work, palladium was chosen to be applied to the slurry method because it has been recently reported (for wet ashed samples) that palladium permits a higher "in furnace" ashing temperature than nickel[20] without premature loss of selenium.

The mechanism of selenium stabilization and release is not completely understood. It is believed that the selenium is trapped through the ashing stage as a metal selenide which has a much higher melting and boiling point than the selenium compounds listed in Table 3.1, and is finally released during the high temperature atomization stage. Matsumoto et al.[24] have reported that electron probe micro-analysis studies indicate that selenium and palladium exist on the surface of the graphite tube in a 1:1 mole ratio.

In developing a slurry method for selenium, several factors were investigated. 1. The possibility of using the same "in furnace" oxygen ashing procedure as the one used by Fietkau[30] was investigated. 2. The applicability of a palladium matrix for the slurry method was checked.

3. The often mentioned spectral interference due to the presence of iron in the sample[16,26] was verified.

Experimental

Apparatus.

A Brinkmann Polytron homogenizer (Brinkmann Instruments, Westbury, NY) with a PTA 20 titanium probe was used for sample homogenization. A Varian GTA - 95 graphite tube atomizer (Varian - Techtron Instrument division., Palo Alto, CA) provided microprocessor controlled heating and gas flows. The GTA - 95 also controlled an autosampling robot which could be programmed to perform dilutions of a premixed standard and could accurately deliver varying amounts of a blank, a modifier, a standard and a sample into the graphite tube. Pyrolytically coated graphite tubes were used for all analyses.

All spectral measurements were performed using a Varian AA - 875 atomic absorption spectrophotometer equipped with a deuterium - arc background corrector. The integrated digital absorbances were taken directly off this instrument. The absorbance profiles versus time were superimposed over the temperature ramp curve and plotted on the CRT screen of the GTA - 95. These were printed using an Axiom TX - 500 video printer (Axiom Corp., San Fernando, CA).

Procedure.

All the glassware and polyethylene bottles were acid

washed prior to use. This was done by keeping them filled with 20% v/v hydrochloric acid for two days followed by 20% v/v nitric acid for two more days. They were then air dried after thoroughly rinsing with distilled deionized water. Distilled deionized water was obtained by deionizing the distilled water using a pair of Barnstead cation exchange columns (Syborn Corp., Boston, MA.).

All dilutions were performed either by volume using volumetric glassware or by weight using a top loading electronic balance. Aqueous selenium standards were prepared by diluting a 1000 ppm Fisher atomic absorption standard (Fisher Scientific Co., Fairlawn, NJ) using distilled deionized water. Fresh standard solutions were prepared daily and discarded at the end of each day. The palladium modifier was prepared by dissolving hydroxylamine hydrochloride (MCB Manufacturing chemists inc., Cincinnati, OH.) in a 1000 ppm palladium standard (5% Hcl matrix) (Spex Industries inc., Edison, NJ.). The resulting solution was 980 ppm palladium and 2% hydroxylamine hydrochloride.

A clean 8 oz square glass bottle was zero tared on a top loading balance with 0.01 g readability. Approximately 30 g of beef liver was placed in it and its exact weight was accurately noted. Without hitting the

tare bar again, distilled deionized water was added to bring up the total weight to exactly 2.000 times the initial weight of the meat product (about 60.00 g). The contents were then homogenized for 2 minutes at top speed using a Brinkmann Polytron homogenizer fitted with a titanium probe. The homogenizer probe was then rinsed, during a low speed operation in three separate portions of distilled deionized water. The above procedure resulted in a 50 % meat slurry. It was divided into two portions.

About 20 g of the first portion was transferred into a clean, zero tared polypropylene bottle on a top loading balance using a clean Pasteur pipet. Without hitting the tare bar, distilled deionized water was added to this until the total weight was about 100 g (exactly five times the weight of the 50 % slurry). The polypropylene bottle was then capped and shaken for about 5 seconds. The resulting slurry was 10% in meat solids.

The second portion was used for wet ashing. About 5 g of the 50% slurry was placed in a 100 mL pyrex glass beaker. Approximately 20 g of Baker Instra - analyzed nitric acid (for trace metal analysis) (J.T.Baker Chemical Co., Phillipsburg, NJ) was added to this. The mixture was covered with a watch glass and allowed to remain at room temperature overnight (about 12 hours). It

was then heated at about 75 ^{o}C with a glass hook propping the watch glass until the total weight of the contents of the beaker was reduced to approximately 5 g or less. The contents were then cooled and diluted by weight using distilled deionized water. NBS # 1568 - Rice flour (U.S.Dept. of Commerce, The National Bureau of Standards, Washington D.C.) was also wet ashed similarly for comparison.

Several different operating conditions were investigated by varying the parameters such as heating profiles, gas flows, sample and modifier volumes to obtain optimal absorption profiles and reduced background. All Analyses were performed using the standard additions technique. NBS standards were analyzed to validate the wet ashing procedure and the analyses.

Results and Discussion

Oxygen Ashing.

Fietkau[30] determined that the background absorbance produced by 10 uL of 10% beef liver slurry was between 0.2 and 0.3 for wavelengths ranging from 300 nm down to 220 nm. It is believed that the background absorbance is partly due to molecules present in the matrix such as alkali halides and partly due to scattering of light by particulate materials released during the atomization process (residual smoke). However, the background absorbance increased very rapidly to about 1.0 absorbance unit as one went from 220 nm down to 200 nm. This is readily explained by the fact that the magnitude of Rayleigh scattering is inversely proportional to the fourth power of the wavelength.

Ten microliters of 10% beef liver slurry was atomized by employing the same heating parameters as those used by Fietkau[30] which are listed in Table 3.2. A background absorbance of about 0.9 was obtained at the 196 nm line of selenium. Although commercial deuterium arc background correctors are claimed to be capable of correcting for background absorbances up to 1.5 absorbance units, skewed and sometimes negative absorbance profiles were recorded in the background corrected mode of the AA-875. A quick look at the

Table 3.2

Heating Parameters

GFAA Analysis of Beef Liver

With Oxygen Ashing.

Stage	Time (s)	Temp (^{o}C)	Sheath gas	Flow (L/min)
Dry	45	120	Nitrogen	3.0
Ash	75	600	Air	3.0
Purge (Ash II)	32	300	Nitrogen	3.0
Atomize	3-4	2400	Nitrogen	0.0

spectrum of the D_2 arc continuum source used for background correction (Figure 3.1) indicates that its relative intensity peaks at approximatly 230 nm and drops sharply from there to 190 nm. As a result, background correction efficiency decreases as we go from 230 nm to 190 nm. Besides, the natural levels of selenium in beef liver are low enough that one has to deal with a very large background to signal ratio, especially at this wavelength.

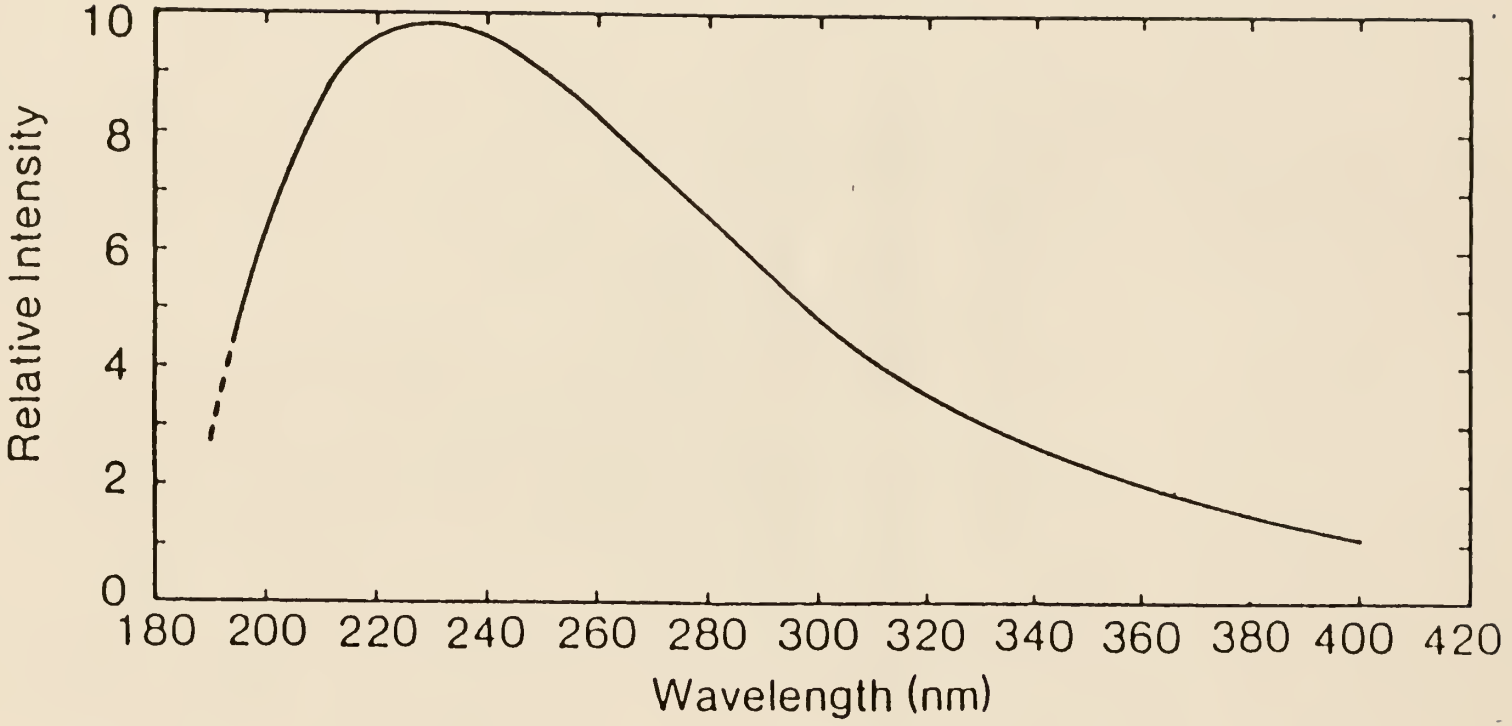
Fietkau[30] observed that ashing temperatures much higher than 600°C during oxygen ashing cause excessive graphite tube erosion. Furthermore, spike recovery studies carried out by spiking the beef liver slurry with known amounts of selenium indicated that the added spikes could not be recovered in a reproducible manner. Under these conditions it was impossible to perform analyses with any great degree of accuracy using oxygen ashing and hence alternative ways of reducing the background and stabilizing the selenium had to be investigated for slurry applications.

Palladium Matrix Modification.

Shrader and Voth-Beach[20] have shown that palladium acts as an effective matrix modifier only if it is present in the reduced metallic state early during the heating cycle so that the selenium can react with it and

Figure 3.1

Emission Spectrum of A Deuterium lamp


Relative Intensity
10
8
6
4
2
0
180 200 220 240 260 280 300 320 340 360 380 400 420
Wavelength (nm)

not be volatilized. In this experiment, the palladium was present in a 5% hydrochloric acid matrix. In order to ensure that the palladium is available in the reduced form, a reducing agent, hydroxylamine hydrochloride was mixed with the palladium modifier. This is often referred to as a "reduced palladium modifier".

Twenty microliters of the modifier was placed in the graphite furnace along with 10 µL of the 10% beef liver slurry. Ashing temperatures as high as 1000 ^{o}C could be used without destabilizing selenium from the palladium surface. Such an unusually high ashing temperature provided for effective ashing of the organic constituents of the beef liver. The ashing scheme consisted of a 30 s ramp and a 30 s hold period and argon was used as the inert gas during the ashing process in order to prolong the tube life. Under these conditions the final non-specific background absorbance during atomization was only 0.5 A which was easily subtracted by the deuterium background corrector.

Voth-Beach and Shrader[20] had earlier found that the presence of oxidizing agents such as nitric acid in high concentrations interfered with analyte stabilization during ashing by preventing the palladium from existing in the reduced metallic state. They observed that this

can be prevented by pre-injecting and heating the palladium modifier to reduce it prior to sample addition. Since the use of nitric acid is eliminated in the slurry method and the natural constituents of beef liver were not found to interfere with analyte stabilization, the slurry procedure can actually serve to eliminate one type of interference (destabilization of the analyte by nitric acid) plaguing the wet ashing method. Pre-injection and heating of the palladium modifier were not found to be necessary with the slurry approach.

Analytical Conditions.

The analytical conditions for selenium determination in beef liver slurry using the reduced palladium modifier are given in Table 3.3. Argon and nitrogen are the most commonly used inert gases. Argon was preferred for this work because it provided better sensitivity for selenium determination. During the atomization stage, the gas flow was stopped in order to reduce gas phase dilution and to maximize the residence time of the analyte atoms in the path of the optical beam inside the furnace. In Table 3.3, steps 1,2,3,5 and 8 are temperature ramps. In all the other steps the temperature is held constant. The "dry" stage consists of steps 1 through 4 when the solvent is evaporated. The ashing stage is from steps 5 through 7. Steps 8 and 9 make up the atomization stage

Table 3.3

Instrumental Parameters

Element: Selenium

Wavelength: 196.0 nm

Bandpass: 1.0 nm

Lamp Current: 7.0 mA

Inert gas: Argon

Step #	Temp (^{o}C)	Time (s)	Gas flow (L/min)
1	75	5.0	3.0
2	90	60.0	3.0
3	120	10.0	3.0
4	120	20.0	3.0
5	1000	30.0	3.0
6	1000	30.0	3.0
7	1000	2.0	0.0
8	2800	0.9	0.0
9	2800	3.1	0.0
10	2800	1.0	3.0

when the signal integration is performed. The suggested atomization temperature[31] for the determination of selenium is 2400 ^{o}C. However 2800 ^{o}C was found to be the atomization temperature necessary to atomize selenium effectively from the palladium matrix. Thus the analytical conditions were developed taking into consideration the fact that all the different stages leading to sample atomization need to be completed effectively in all the different sample matrices.

Spectral interference due to the presence of iron in the samples can cause errors due to over correction in instruments fitted with a deuterium-arc background corrector. Iron lines in proximity of the 196 nm Se line are within the instrument bandpass and cannot be resolved by the monochromaters normally used for atomic absorption spectroscopy. They are, however not within the much narrower selenium absorbance profile. As a result, the iron atoms in the sample are excited by the D_2 lamp and not by the the selenium hollow cathode lamp. So background subtraction can lead to overcorrection and apparent "negative" absorbances. Ten micrograms of iron was atomized along with 10 µL of 10% beef liver and was found to give rise to negative absorbances. However, the natural levels of iron present in the beef liver alone are much less and were not found to interfere with the

selenium determination. Hence, there was no need to use an alternative analytical line such as the 204 nm line of selenium for these samples. Due to higher iron content, blood would most likely cause more difficulty in this regard.

Calibration and Detection Limit.

Standard addition calibration was found to be necessary to determine selenium in both the slurry and the wet ashed samples. This is readily evident from Figure 3.2 where the calibration plots for the determination of selenium in the aqueous, wet ashed and slurry matrices are superimposed on a single graph. The overall mechanism by which the selenium is stabilized in each of these matrices is assumed to be the same. However, the slopes of the three calibration curves are different and unique owing to the differences in the manner and the rates in which the three different types of samples are deposited in the graphite tube, desolvated, ashed and atomized. So using the calibration curve developed for one matrix to determine the analyte in another matrix will lead to incorrect answers. Matrix matching techniques to match the matrices of the samples and the standards have been succesfully carried out in many instances. However it is our opinion that accurate matrix matching for slurries is neither feasible at this

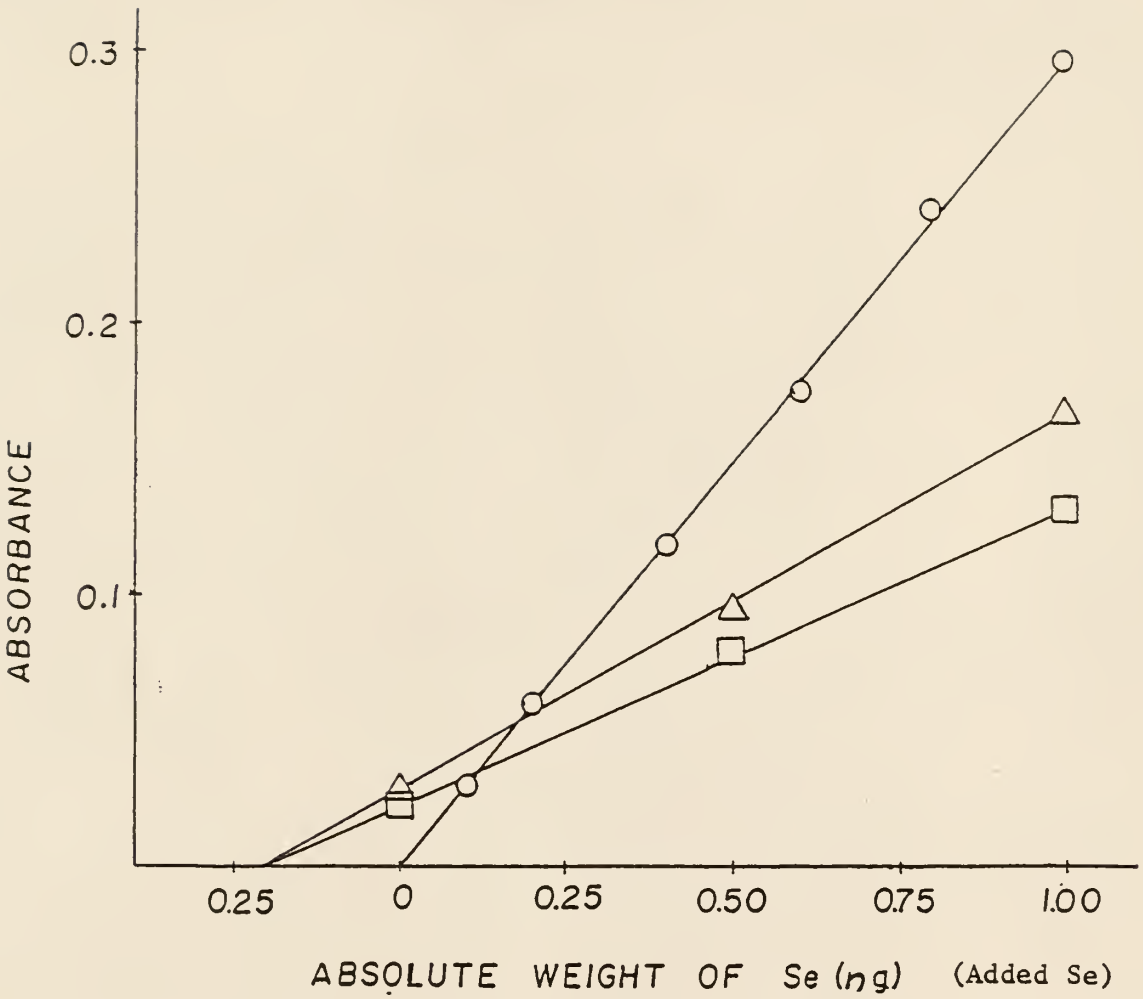
Figure 3.2

Calibration curves for Se determination

□ - 10 uL 10% slurry

△ - 10 uL 10% wet ashed sample

○ - aqueous Se standard


0.3
0.2
0.1
ABSORBANCE
0.25
0
0.25
0.50
0.75
1.00
ABSOLUTE WEIGHT OF Se (ng) (Added Se)

point nor is necessary when the problem of matrix mismatch can be solved easily and effectively by using the method of standard additions which is performed automatically by the sampling system (autosampler robot). In figure 3.2, the reader should note that even though the slope of the curves is different, the extrapolated x-axis intercept (which tells the unknown concentration) is nearly identical for the standard add curves of wet ashed and slurry samples.

Detection limits (three times the standard deviation of the blank) were computed for selenium determination in the aqueous and the slurry phases. The detection limit for selenium in the aqueous phase on an absolute weight basis was determined to be 26 **picograms.** The detection limit in the diluted slurry phase was **13 ppb.** This was based on 10 uL aliquots of a 10% slurry. So the solid phase detection limit in beef liver was determined to be **0.13 ppm.**

Precision.

The precision for selenium determination using the slurry and the wet ashed samples was compared by computing the relative standard deviation for 5 replicates. The relative standard deviations were 15% and 11% for the slurry and the wet ashed samples respectively, as shown in table 3.4. It is important to

Table 3.4

	Solid phase Concentrations (ppm)	
10% Beef liver slurry	0.20 ±0.03 (15% RSD)	
10% Wet ashed beef liver	0.19 ± 0.02 (11% RSD)	
	Expected	Observed
NBS Rice flour (SRM # 1568)	0.4 ± 0.1	0.38 ± 0.02 (5% RSD)

note that these precision data were obtained using background corrected absorbances of 20 ppb Se in the 10% slurry and the corresponding wet ashed solution. These values are only slightly higher than the detection limit of the method which was 13 ppb in the slurry phase. Precision is always at its worst near the limit of detection. Higher analyte levels would most likely result in better precision for both the wet ashed and slurry methods.

Accuracy.

In order to obtain accurate results, care was taken to use clean, acid washed glassware at all times. Slurry and wet ash blanks were prepared by simultaneously carrying distilled deionized water through all the sample preparation steps. The wet ashing procedure and the subsequent GFAA determination were validated by determining the selenium content of NBS rice flour(SRM # 1568). The results are displayed in Table 3.4 and it can be seen that the slurry method provides for accurate determination of selenium in beef liver.

References.

1. B.V. L'vov, Engng.Physics J. USSR 2(2), 44-52 (1959).

2. H. Massman, II Internationales Symposium "Reinststoffe in Wissenschaft and Technik" (Dresden, 1965), s.297, Akademie- Verlag, Berlin (1966).

3. R. Woodriff and R. Stone, Appl. Optics 7, 1337 (1968).

4. K. Saeed, Y. Thomassen and F.J. Langmyhr, Anal. Chim. Acta, 110, 285-289 (1979).

5. T.M. Vickrey and M.S. Buren, Anal. Lett. 13(A17), 1465-85 (1980).

6. R.F. Sanzolone and T.T. Chao, Anal. Chim. Acta, 128(1), 225-7 (1981).

7. W. Slavin and D.C. Manning, Proc. Anal. At. Spectrosc. 5, 243 (1982).

8. C.H. Chung, E. Iwamoto, M. Yamamoto, Y. Yamamoto and M. Ikeda, Anal.Chem. 56(4), 829-31 (1984).

9. B. Welz, G. Schlemmer, U. Voellkopf, Spectrochim. Acta, Part B, 39B(2-3), 501-10 (1984).

10. X. Shan and K. Hu, Talanta, 32(1), 23-6 (1985).

11. U. Voellkopf, Z. Grobenski, R. Tamm and B. Welz, Analyst(London), 110(6), 573-7 (1985).

12. S.A. Lewis, N.W. Hardison and C. Veillon, Anal. Chem., 58(6), 1272-3 (1986).

13. M.S. Droessler and J.A. Holcombe, Can.J.Spectrosc., 31(1), 6-10 (1986).

14. A. Cedergren, I. Lindberg, E. Lundberg, D.C. Baxter and W. Frech, Anal. Chim. Acta, 180, 373-88 (1986).

15. D.L. Styris, Fresenius' Z. Anal. Chem., 323(7), 710-15 (1986).

16. P.L.H. Jowett and M.I. Banton, Anal. Lett., 19(11-12), 1243-58 (1986).

17. L.M. Voth-Beach and D.E. Shrader, Spectroscopy, 1(10), 49-50, 52, 55-9 (1986).

18. G. Schlemmer and B. Welz, Spectrochim. Acta, Part B, 41B(11), 1157-65 (1986).

19. G. Schlemmer and B. Welz, J. Anal. At. Spectrom., 2(1), 119-124 (1986).

20. L.M. Voth-Beach and D.E. Shrader, J. Anal. At. Spectrom., 2(1), 45-50 (1987).

21. K. Saeed, J. Anal. At. Spectrom., 2(2), 151-5 (1987).

22. C.A. Shand and A.M. Ure, J. Anal. At. Spectrom., 2(2), 143-9 (1987).

23. J. Dedina, W. Frech, I. Lindberg, E. Lundberg and A. Cedergren, J. Anal. At. Spectrom., 2(3), 287-91 (1987).

24. J.E. Teague-Nishimura, T. Tominaga, T. Katsura and K. Matsumoto, Anal. Chem., 59(13), 1647-51 (1987).

25. A.J. Krynitsky, Anal. Chem., 59(14), 1884-1886 (1987).

26. B. Sampson, J. Anal. At. Spectrom., 2(5), 447-50

(1987).

27. J. Dedina, W. Frech, A. Cedergren, I. Lindberg and E. Lundberg, J. Anal. At. Spectrom., 2(5), 435-9 (1987).

28. R.C. Fry and M.B. Denton, Anal. Chem., 49(9), 1413-1416 (1977).

29. N. Mohamed, R.M. Brown,jr. and R.C. Fry, Appl. Spectrosc., 35(2), 153-163 (1981).

30. R. Fietkau, Ph.D dissertation , Kansas State University, 1986.

31. E. Rothery, Analytical Methods for Graphite Tube Atomizers, Varian Techtron Pty. Limited, Mulgrave, Victoria, Australia.

Chapter 4

Stability of Chromium (III) Lignosulfonate Against Oxidation by Chlorinated Tap Water

Introduction

Chromium (VI) is a known environmental toxin which is regulated by the United States Environmental Protection Agency (EPA)[1]. In contrast, Chromium (III) is a known nutrient which is environmentally benign. However, several authors have recently shown that Cr (III) may be environmentally oxidized to the more harmful hexavalent form. One environmental oxidizing agent of particular concern is free chlorine in tap water. One study has shown chlorinated tap water to be capable of oxidizing Cr (III) to Cr (VI)[2].

As a result of the studies mentioned above, the EPA has defined the legal limit for drinking water in terms of total chromium instead of just the toxic form (Cr (VI)). The limit is currently 0.05 ppm total chromium, but the agency is considering raising this value to 0.12 ppm. As a further result of the above mentioned oxidation studies, the EPA has also been reluctant to approve landfill waste disposal of chromium (III) lignosulfonate byproducts (silvichemicals) from the wood pulp industry.

Unfortunately, at least four major problems arise with trying to extrapolate from the above mentioned Cr (III) oxidation studies (on which the EPA decision was based) in indicting Cr (III) lignosulfonate as a

potential precursor to the toxic form (Cr (VI)). First, the Cr (III) oxidation studies involving tap water were conducted at elevated chlorine levels (>2 ppm) rather than the normal chlorine concentration (nominally 1 ppm, with a typical range of 0.8 - 1.2 ppm, and an extreme range of 0.5 - 1.5 ppm free residual chlorine). The rate law for Cr (III) oxidation has not been reported; however, if that law should eventually prove to be nonlinear in chlorine concentration, the EPA indictment of Cr (III) lignosulfonate based on studies conducted at elevated (rather than normal) chlorine levels in tap water would be found premature and perhaps unfair.

Second, the Cr (III) oxidation studies were conducted over a time period of only 20 hours and may be kinetically hindered. It is therefore possible that the results may not be representative of an equilibrium or long term environmental situation.

Third, Cr (III) oxidation was only studied in a narrow range of chromium concentration (0.2 - 0.5 ppm Cr (III)). A wider range of Cr (III) concentration should be studied to cover the distinct possibility of unusual (nonlinear) kinetic behavior.

Finally, the oxidation studies unfortunately only included trivalent chromium in its chloride form. No

study has been reported on the tap water oxidation of Cr (III) in the lignosulfonate form. A major difference between the two forms of trivalent chromium might well occur in terms of oxidation potential. In spite of the known tendency of Cr (III) chloride to be oxidized, it is entirely possible that the chelating effect of lignosulfonate could stabilize the +3 state of chromium against oxidation by chlorinated tap water. This could occur via a shift in Cr (III) oxidation potential.

The purpose of this investigation was to study the oxidation of Rayonier Chromium Lignosulfonate (UNICAL) by chlorinated tap water in order to determine whether the lignosulfonate group is capable of stabilizing the harmless +3 state of Cr against oxidation to the toxic form (Cr (VI)). The investigation also includes an evaluation of the reactivity of tap water at both elevated and normally encountered chlorine levels (rather than just elevated levels). A wider range of Cr concentration is studied, and considerably longer reaction times are employed than have previously been reported for Cr (III) oxidation by chlorinated tap water.

The present study involved treating solutions of chromium (III) lignosulfonate of widely differing concentrations with varying amounts of calcium hypochlorite. The reactions were monitored for production

of any chromium (VI) due to possible oxidation of the Cr(III) lignosulfonate by free chlorine (from dissolved $Ca(OCl)_2$). Chromium(VI) production was measured spectrophotometrically using the well known diphenylcarbazide color forming reagent (1). The free chlorine concentration was also monitored during the course of each reaction. Free chlorine determination was done spectrophotometrically using the orthotolidine reagent (1).

EXPERIMENTAL

Apparatus.

A Bausch and Lomb Spectronic 2000 (Bausch and Lomb, Systems Div., Rochester, NY) spectrophotometer was used to make all spectrophotometric measurements. A matched pair of 1.0 cm glass cuvettes were used.

Reagents.

All concentrated stock solutions were made by volume in acid washed volumetric glassware using distilled deionized water. Any dilute chromium containing solutions (including all test samples undergoing reaction with chlorine) were prepared and stored in polypropylene bottles.

Chlorine stock solution. The stock solution used to chlorinate the test samples was prepared by dissolving 1.45 g of calcium hypochlorite (Fisher Scientific Co., Fairlawn, NJ) in 1 liter of distilled deionized water in a volumetric flask. This solution was then transferred into a polypropylene bottle, capped tightly and stored in the dark. Since the solution was not prepared using "chlorine-demand free" water and was not very stable, the free residual chlorine concentration of the stock solution could not be accurately calculated from initial weight/volume data. Hence, the stock was diluted and its

free residual chlorine content determined "in situ" by the orthotolidine method as and when needed.

Orthotolidine reagent. The orthotolidine reagent for chlorine determination was prepared by dissolving 1.35 g of orthotolidine dihydrochloride (Fisher Scientific Co., Fairlawn, NJ) in 350 mL distilled deionized water and 150 mL concentrated hydrochloric acid and then diluting it to 1 liter with distilled deionized water. It was stored in an amber glass bottle.

Phosphate buffer stock. 22.86 g of anhydrous disodium hydrogen phosphate (Mallinckrodt Chemical Works., St. Louis, MO) and 46.16 g of anhydrous potassium dihydrogen phosphate (J.T.Baker Chemical Co., Phillipsburg, NJ) were dissolved in 1 liter distilled deionized water to give a phosphate buffer which was used to prepare standards for the chlorine determinations.

Permanent "chlorine" standards. 0.775 g of potassium dichromate (J.T.Baker Chemical Co., Phillipsburg, NJ) and 2.325 g of potassium chromate (Mallinckrodt Chemical works., St. Louis, MO) were dissolved in 500 mL of a phosphate buffer obtained by a five fold dilution of the original phosphate buffer stock solution. Although this solution contains no chlorine, it is known to produce the same absorbance at 435 nm as would a 10 ppm free residual chlorine solution (1). The buffered potassium

chromate/dichromate solution was therefore adopted as a pseudo-chlorine standard. This was diluted to give a series of permanent "chlorine" standards. This is a well known, standard method of free residual chlorine determination (1).

Chromium (III) and Chromium (VI) solutions. Standard Cr (III) solution was prepared by dissolving chromium chloride hexahydrate (Fisher Chemical Co., Fairlawn, NJ) in distilled deionized water. Standard Cr(VI) solutions were prepared from both potassium chromate (Mallinckrodt Chemical Works., St. Louis, MO) and potassium dichromate (Fisher Scientific Co., Fairlawn, NJ) for comparison. Solutions of chromium lignosulfonate were prepared by dissolving chromium (III) lignosulfonate (Unical-4.5% Cr(III), ITT Rayonier Inc., Shelton, WA) in distilled deionized water. Chromium-free lignosulfonate solutions were also prepared for comparison purposes by dissolving Cr-free lignosulfonate (Orzan, ITT Rayonier Inc., Shelton, WA) in distilled deionized water.

Diphenylcarbazide Reagent. The diphenylcarbazide reagent (DPC) was prepared by first dissolving 0.2 g of diphenylcarbazide (1,5 - Diphenylcarbohydrazide) in 100 mL ethyl alcohol followed by the addition of 40 mL concentrated sulfuric acid and 360 mL distilled deionized

water. It was stored in the dark in an amber bottle.

Procedure

The test solutions were prepared by rapid dilution and mixing of the stock solutions by weight using a top loading electronic balance. The solutions were made in 125 mL polypropylene bottles, capped tightly and stored in the dark. Sub-samples were drawn periodically to determine Cr(VI) and free chlorine. Four sub-sample aliquots were drawn from each sample. One was treated with the diphenylcarbazide reagent while another was used as a blank. Of the remaining two aliquots, one was a blank and the other was reserved for an orthotolidine reaction.

Notes on the analytical methods are included in appendix A. For initial and free residual chlorine determinations, 5 mL of each of the samples were treated with 0.25 mL of orthotolidine reagent and their maximum absorbances at 435 nm were determined. Calibration curves were also plotted by measuring the permanent "chlorine" standards at 435 nm.

For the Cr(VI) determination, 5 mL of each of the solutions were treated with 0.25 mL of the diphenylcarbazide reagent and their maximum absorbances were measured at 540 nm. In this case also calibration

curves were plotted using Cr(VI) standards.

A wide range of chromium (III) lignosulfonate solutions from 0.1 ppm to 50.0 ppm were investigated. Solutions of calcium hypochlorite ranging from 0.5 ppm to 5.0 ppm combined available chlorine were used to oxidize the chromium (III) lignosulfonate. These reactions were monitored for Cr (VI) production and free residual chlorine content at regular intervals over a period of 4 to 6 weeks.

Long Term Reaction with Normal Tap Water.

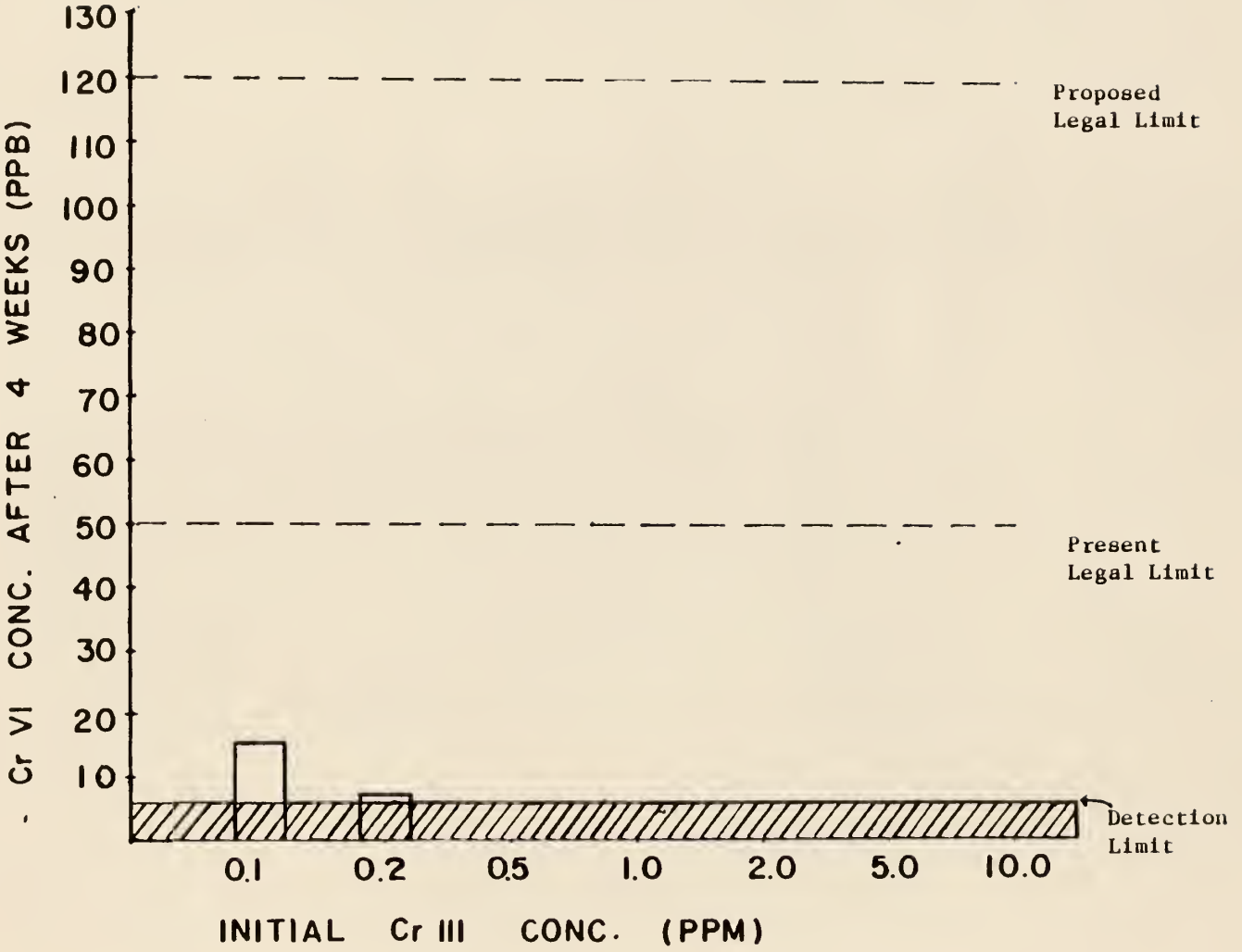
As will be shown later in this chapter, any significant Cr (III) lignosulfonate reaction with chlorinated tap water essentially reaches completion within four weeks (at room temperature). Four weeks will therefore be considered the equilibrium or long term case.

The single most important case to consider is that of long term reaction of Cr (III) lignosulfonate with free chlorine at the concentration occurring in normal tap water (nominally 1.0 ppm free residual chlorine), because that will provide the most realistic insight into the environmental fate of this compound. In sharp contrast to the earlier studies based on Cr (III) chloride and elevated chlorine levels (e.g. 3 ppm[2]), Figure 4.1 shows almost no detectable reactivity of Cr (III) lignosulfonate with 1.0 ppm free residual chlorine in tap water over a wide range of Cr (III) concentration. This occurs in spite of the fact that the Cr (III) lignosulfonate reactions of Figure 4.1 were allowed to proceed for four weeks, whereas the earlier reported reactivity of Cr (III) chloride occurred within 20 hours.

The data of Figure 4.1 indicate that the long term environmental oxidation of Cr (III) lignosulfonate by

Figure 4.1

Oxidation of Cr(III) Lignosulfonate by 1.0 ppm Free Chlorine.


130
120
110
100
90
80
70
60
50
40
30
20
10
- Cr VI CONC. AFTER 4 WEEKS (PPB)
Proposed
Legal Limit
Present
Legal Limit
Detection
Limit
0.1
0.2
0.5
1.0
2.0
5.0
10.0
INITIAL Cr III CONC. (PPM)

normal chlorinated tap water is of no significant concern. At Cr (III) lignosulfonate concentrations above 0.2 ppm, no detectable toxic Cr (VI) is produced whatsoever (all Cr (VI) values are <0.006 ppm).

Cr (III) lignosulfonate concentrations at or below 0.05 ppm were not investigated for the simple reason that, even if reaction with chlorine were complete, the starting amount of Cr (III) would be too small for the final level of Cr (VI) to reach or exceed the present legal limit (0.05 ppm). Furthermore, EPA is considering raising the permissible chromium level to 0.12 ppm. Consequently, from an environmental safety standpoint, the only significant Cr (III) lignosulfonate concentrations to examine in further detail fall in the quite narrow range between about 0.1 and 0.2 ppm.

In the range 0.1 - 0.2 ppm Cr (III) lignosulfonate, Figure 4.1 shows that long term reaction with normal tap water (1.0 ppm free chlorine) will produce a minimal (but harmless) conversion to Cr (VI). The levels of Cr (VI) produced in this case (0.016 ppm and 0.007 ppm) are however barely detectable and are well below the current and proposed legal safe limits (0.050 and 0.120 ppm, respectively) for drinking water. As far as the legal question of environmental safety is concerned, our investigation to this point has refuted the possibility

of Cr (III) lignosulfonate oxidation by normal chlorinated tap water.

Interestingly, Figure 4.1 gives some indication that: the higher the Cr (III) Lignosulfonate concentration, the less likely any Cr (VI) will be produced by oxidation with free chlorine. This is certainly not what we would have expected, and it would seem to suggest that large amounts of Cr (III) lignosulfonate are even safer to dump for tap water interaction than small amounts!

Reaction Mechanism.

One possibility is that the large organic section of the Cr (III) lignosulfonate molecule is reactive toward free chlorine and consumes it before reaction with Cr (III) can occur. This could be a protective mechanism against oxidation of Cr (III) by chlorinated tap water.

If the reaction of free chlorine with the lignosulfonate section of the Cr (III) lignosulfonate molecule is faster or proceeds with a more favorable equilibrium constant than reaction with Cr (III), we would find a distinct two step oxidation mechanism at work. The first step would be oxidation of the organic part of the molecule by free chlorine. If the lignosulfonate normality exceeds the free chlorine

concentration, then the chlorine would be consumed and would not be available for the second step of the oxidation mechanism (oxidation of Cr (III)). If the lignosulfonate normality is below the free chlorine concentration, then some excess free chlorine would remain after oxidation of lignosulfonate. This excess free chlorine would then be available to complete the slower oxidation of Cr (III).

If the above mechanism occurs and a fixed amount of free chlorine is studied (e.g. 1.0 ppm), we would predict an interesting relationship between Cr (VI) production and the starting amount of Cr (III) lignosulfonate. At high starting amounts of Cr (III) lignosulfonate where the number of equivalents of lignosulfonate exceeds the number of equivalents of chlorine, the chlorine would be entirely consumed in step 1 of the oxidation. In this case step 2 (oxidation of Cr (III)) could not proceed and no detectable Cr (VI) would be produced. At the other extreme (low starting amounts of Cr (III) lignosulfonate) where the number of equivalents of lignosulfonate are exceeded by the fixed number of chlorine equivalents, enough excess free chlorine would remain after completing step 1 (lignosulfonate oxidation) for further reaction with Cr (III) (step 2). In this case we would see some Cr (VI) production, **but the amounts would be limited by**

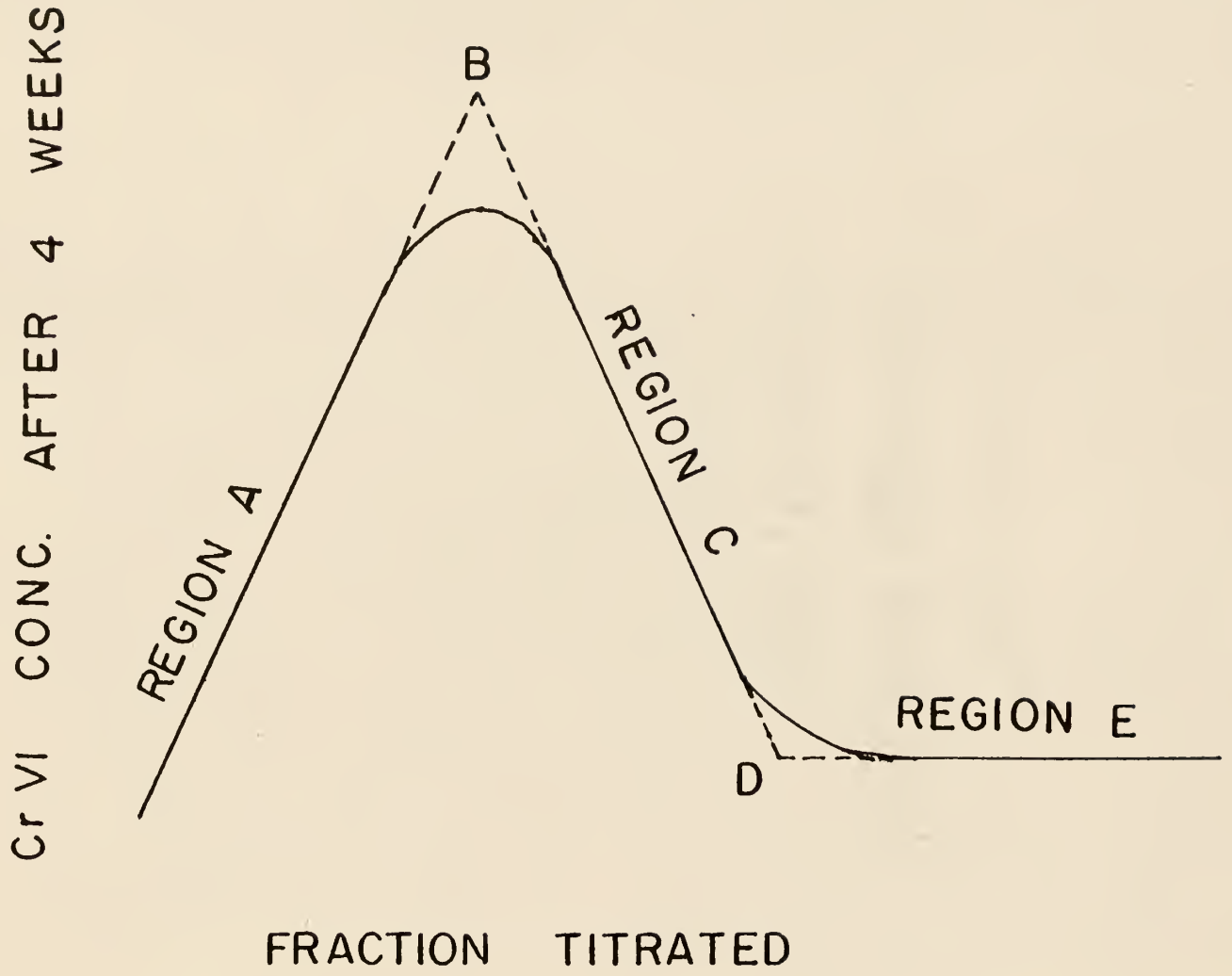
the small starting amount of Cr (III). Prediction of these small amounts would proceed as follows.

If a "titration" of a fixed free chlorine sample (e.g. 1.0 ppm) with increasing amounts of standard Cr(III) lignosulfonate were to be carried out (using Cr(VI) production to monitor the course of the titration), our two step redox mechanism would predict two equivalent points. The shape of the titration curve would be nonlinear in one region and would appear as in Figure 4.2 with all the points on the curve measured after four weeks reaction time.

Early in the titration (at very low amounts of Cr (III) lignosulfonate added), the chlorine would be in excess over both the lignosulfonate and the Cr (III). In this region (region A, Figure 4.2), both steps of the oxidation would occur and some Cr (VI) would be detected. In region A, adding increments of Cr (III) lignosulfonate would lead to increased Cr (VI) production (as long as the free chlorine remains in large excess over both the lignosulfonate and the Cr (III)). In this region (A), the percent conversion of Cr (III) to Cr (VI) should be high and nearly independent of the initial chlorine content. When corrected for dilution, the Cr (VI) concentration at this point should equal the original concentration of Cr (III) added, no matter what the free

Figure 4.2

A model for the **"titration"** of a **fixed free chlorine sample** with **standard Cr(III) lignosulfonate.**


Cr VI CONC. AFTER 4 WEEKS
B
REGION A
REGION C
REGION E
D
FRACTION TITRATED

chlorine level is (unless the conditional equilibrium constant for Cr (III) oxidation is not sufficiently large and the entire experiment is too dilute).

It should be noted that even though the percent conversion of Cr (III) to Cr (VI) will be high in region A, the absolute levels of Cr (VI) produced will be low due to the low starting amounts of Cr (III) available throughout this region and the dilute nature of the titration as a whole.

The first equivalence point would occur when the number of chlorine equivalents equals the sum of the number of added Cr (III) and lignosulfonate equivalents. (Note: Cr (III) lignosulfonate contains only 4.5% chromium, and the lignosulfonate moiety is likely to contain more than one oxidizible site, so the number of lignosulfonate equivalents probably increases faster than the number of Cr (III) equivalents as the titration proceeds. Point B should therefore be reached at relatively low concentrations of added Cr (III) (in the lignosulfonate form)). At this moment in the titration (point B, Figure 4.2), the chlorine would be fully consumed except for a residual amount due to the finite conditional equilibrium constant.

Past point B, we are adding excess Cr (III)

lignosulfonate (region C) with no further chlorine oxidant available. Ordinarily, the Cr(VI) would reach a constant level in region C of Figure 4.2. However, it must be remembered that if the oxidation of Cr(III) (step 2) is reversible and step 1 of the chlorine reaction (lignosulfonate oxidation) is more favorable, region C represents a lignosulfonate excess which would displace some of the Cr(VI) produced earlier in regions A and B. This is especially true if the titration were carried out in parallel instead of sequential incremental format; namely, if multiple aliquots of the starting chlorine sample are spiked separately with different amounts of Cr(III) lignosulfonate and all reacted simultaneously for four weeks. In this case, the shape of region C would not even depend on the reversibility of Cr(III) oxidation. It would simply decrease steadily (see Figure 4.2, region C) until the second equivalence point (D) is reached.

In a parallel aliquot titration, the fixed amount of chlorine has been depleted at all points in region C, but the lignosulfonate moiety is not yet in excess. (Only the sum of the lignosulfonate and the Cr(III) is in excess over the chlorine). Since the lignosulfonate moiety reacts with chlorine more strongly than does Cr(III), region C will be characterized by decreasing Cr(VI)

production. The lignosulfonate is either displacing Cr(VI) produced earlier in the titration, or the chlorine simply reacts first with all available lignosulfonate (step 1) leaving a steadily decreasing excess available for the less favorable Cr(III) oxidation in step 2. In either case, the Cr(VI) concentration in region C would decrease with increasing additions of Cr(III) lignosulfonate (even though more Cr(III) has been added). Throughout region C, the sum of the Cr(III) and lignosulfonate equivalents exceeds the original chlorine content, but the number of lignosulfonate equivalents alone does not.

At point D, the second equivalence point in Figure 4.2, the number of lignosulfonate equivalents finally matches the original chlorine content, and the chlorine reacts preferentially and exclusively with the lignosulfonate moiety (leaving no remaining chlorine excess to react with Cr(III)). At this point in the titration, no detectable Cr(VI) should be observed, and the free chlorine content should be virtually exhausted (except for residual amounts due to a finite equilibrium constant).

Region E of Figure 4.2 represents large excesses of both the lignosulfonate moiety and of Cr(III). The more

favorable reactivity and larger concentration excess of the lignosulfonate moiety will prevail indefinitely over the Cr(III), so no further Cr(VI) production should occur.

Figure 4.2 is just a model. If we now look back to the original data of Figure 4.1, we can begin to obtain some experimental data to verify the proposed two step oxidation by seeing how well the data conform to the model. In comparing Figures 4.1 and 4.2, we see that the data for normal chlorinated tap water follows the predictions of the model in regions C, D and E. of Figure 4.2. (The titration of Figure 4.1 simply did not include any data in regions A and B.)

Although the first equivalence point was missed, Figure 4.1 for normal tap water shows decreasing Cr(VI) production at points immediately prior to the second equivalence point (D). For points past the second equivalence point of the titration, increasing Cr(III) lignosulfonate concentrations should result in no detectable Cr(VI) production whatsoever (region E). Figure 4.1 shows exactly this behavior.

The second equivalence point (D) for 1.0 ppm chlorine occurs after addition of approximately 0.2 ppm Cr(III) (lignosulfonate form). Before attaching much significance to this approximate equivalence point in

terms of possible reaction stoichiometry, it should be remembered that the units here are w/w, the x-axis scale of Figure 4.1 is nonlinear, the entire "titration" of Figure 4.1 is conducted in a y-axis region near or below the Cr(VI) detection limit, and the lignosulfonate moiety is in large excess over the Cr(III) in this molecule. In fact, Cr(III) lignosulfonate contains only about 4.5% chromium.

The fixed chlorine content of the tap water sample used in the "Cr(III) lignosulfonate titration" of Figure 4.1 is such that the first equivalence point was missed and the second equivalence point is reached very quickly. This result is certainly desirable from the standpoint of environmental safety and is accurately representative of normal tap water, but it is not optimal for verification of our proposed two step redox mechanism.

Region E past the second equivalence point D is well enough represented by Figure 4.1, but more points are needed early in the titration (especially in the dilute range <0.1 ppm Cr(III) lignosulfonate) to experimentally verify the existence of region A and the first equivalence point B predicted by the model of Figure 4.2. To improve the situation and permit better verification of our proposed two step redox mechanism, this region of

the "titration" can be investigated either by titrating with more dilute Cr(III) lignosulfonate or by repeating the titration with the same Cr(III) lignosulfonate concentrations and a larger starting concentration of free chlorine (elevated beyond the level normally found in tap water). The latter approach was selected in order to increase the y-axis "gain" of the titration and decrease the uncertainty incurred with measuring Cr(VI) near the detection limit.

Long Term Reaction with Exaggerated Free Chlorine Levels.

The result of increasing the chlorine level of the titration without changing the size of the Cr(III) lignosulfonate increments should be: 1.) to shift the equivalence points to the right (toward higher Cr(III) levels), 2.) to produce a steeper rate of decline in region C, 3.) to show a first equivalence point B, and 4.) to produce an initial region A of rising Cr(VI) production which is relatively independent of chlorine content. Figures 4.3 - 4.5 show exactly this behavior. Figure 4.3 was for only slightly more chlorine (1.5 ppm) than Figure 4.1 (1.0 ppm), so it does not show all of the desired benefits. Figure 4.4 at 2.0 ppm chlorine would have benefitted from a few more data points to define the curve, but it definitely shows the beginnings of a first

Figures 4.3,4.4 and 4.5.

Oxidation of Cr(III) lignosulfonate by free chlorine.

Figure #	Added Free Chlorine
4.3	1.5 ppm
4.4	2.0 ppm
4.5	5.0 ppm

Figure 4.3

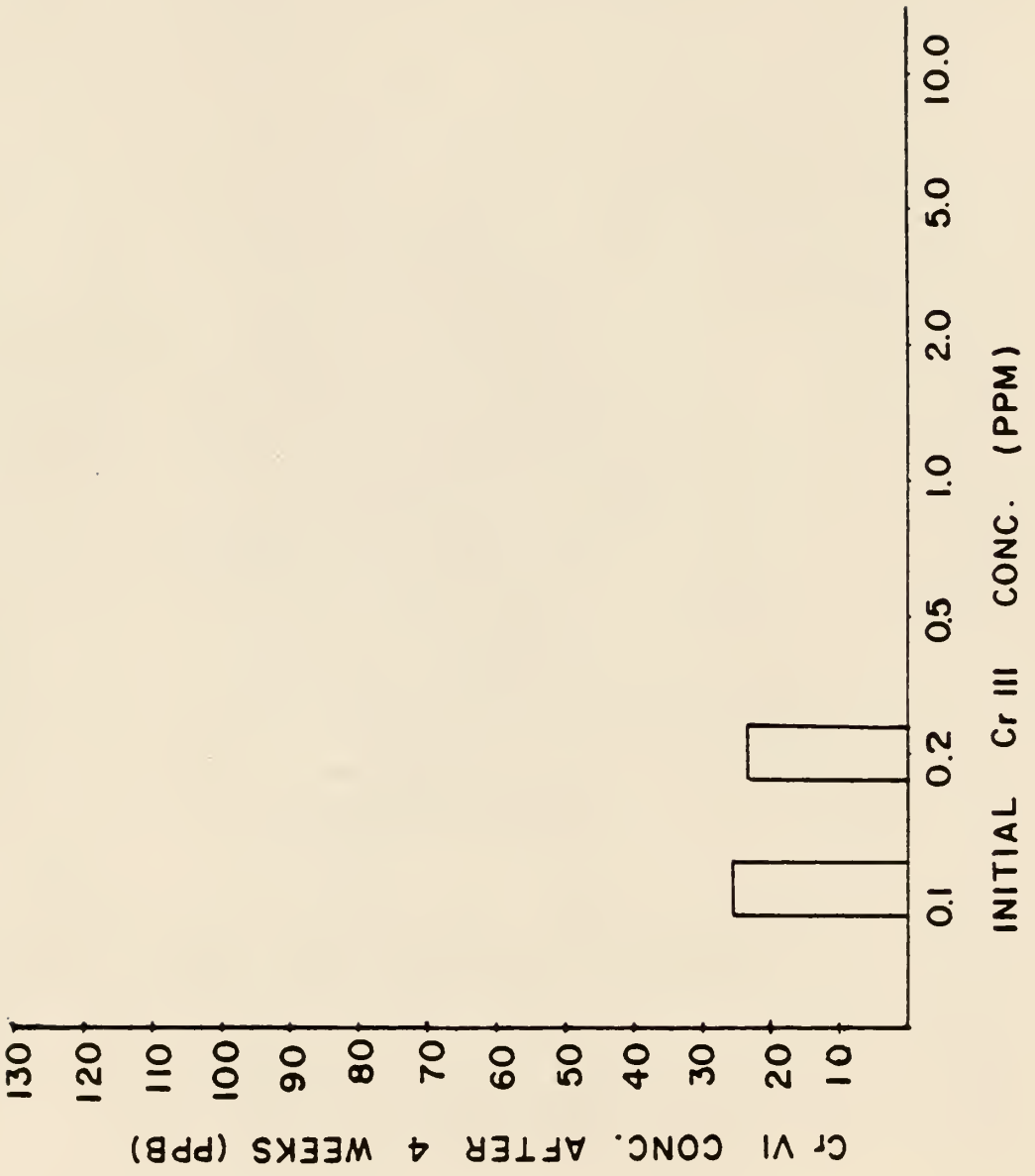

Cr VI CONC. AFTER 4 WEEKS (PPB)
130
120
110
100
90
80
70
60
50
40
30
20
10
0.1
0.2
0.5
1.0
2.0
5.0
10.0
INITIAL Cr III CONC. (PPM)

Figure 4.4

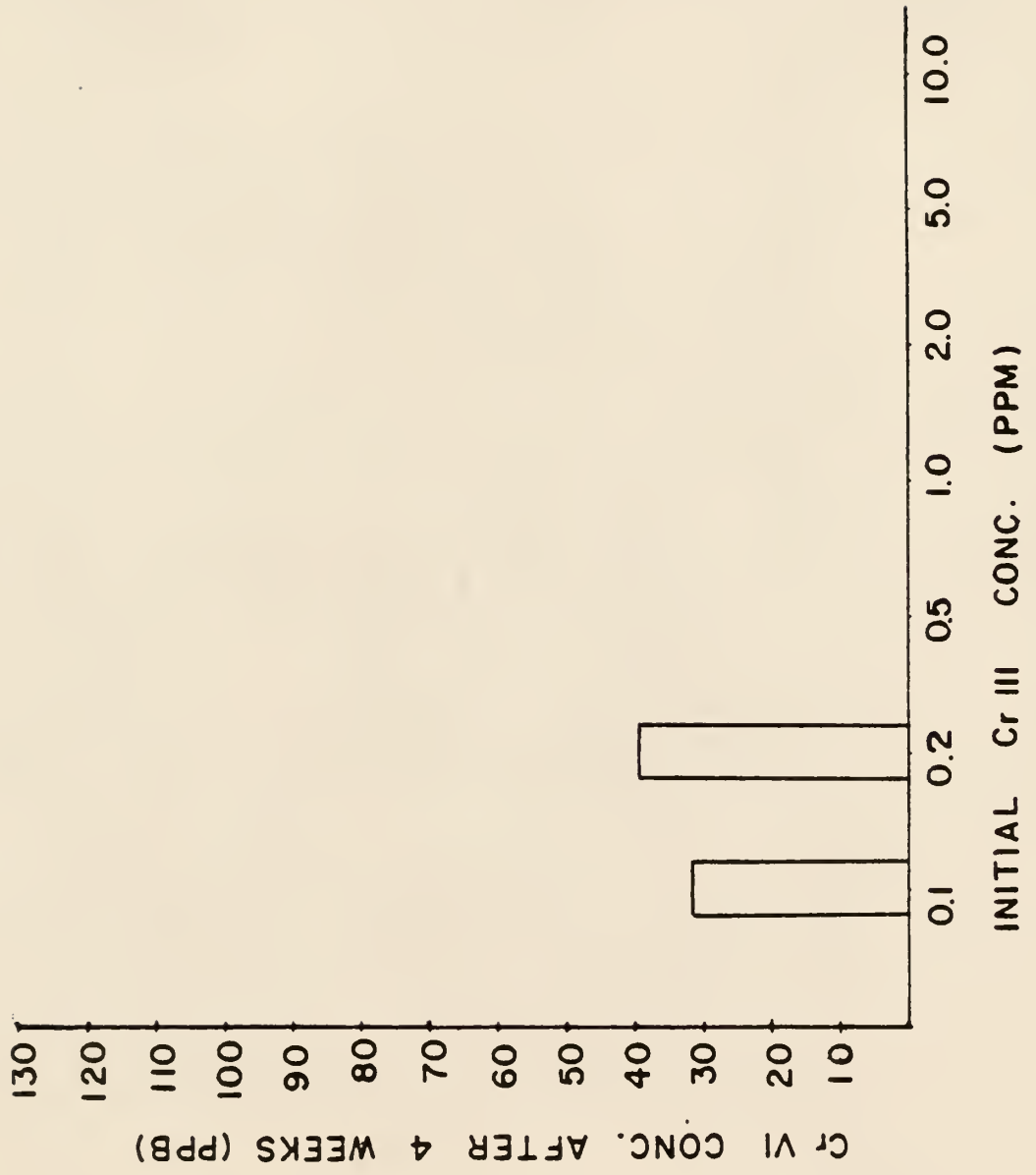
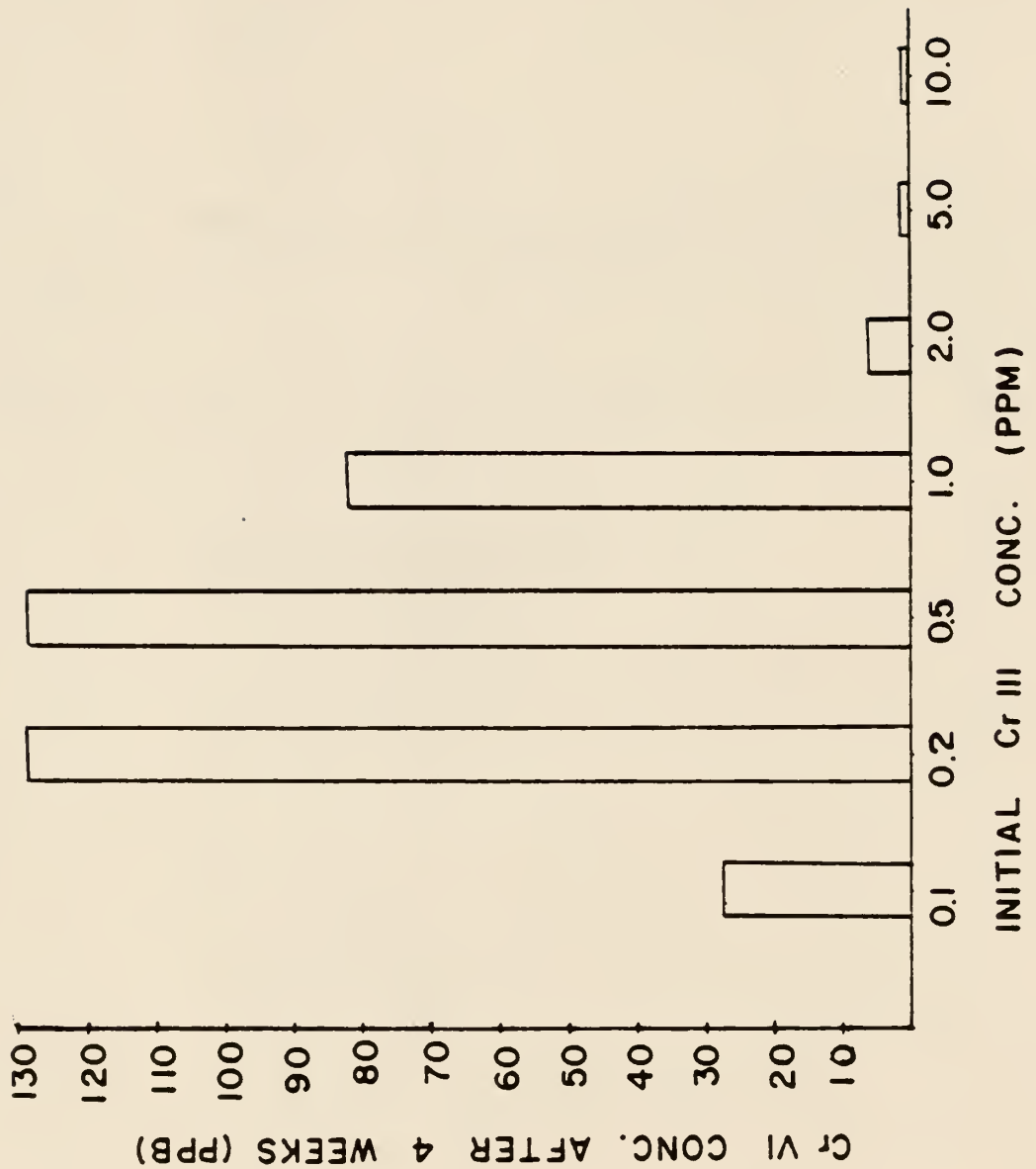

Cr VI CONC. AFTER 4 WEEKS (PPB)
130
120
110
100
90
80
70
60
50
40
30
20
10
0.1
0.2
0.5
1.0
2.0
5.0
10.0
INITIAL Cr III CONC. (PPM)

Figure 4.5


Cr VI CONC. AFTER 4 WEEKS (PPB)
130
120
110
100
90
80
70
60
50
40
30
20
10
0.1
0.2
0.5
1.0
2.0
5.0
10.0
INITIAL Cr III CONC. (PPM)

equivalence point B and a region A of increasing Cr(VI) production. Figure 4.5 is the one with enough chlorine (5.0 ppm) to overcome most of the deficiencies of the earlier titrations. It agrees quite well with all aspects of the model of Figure 4.2. In Figure 4.5, both equivalence points are clearly visible, and they have been shifted toward the right, as expected. Region A where Cr(VI) production should be on the rise is clearly apparent in Figure 4.5. Furthermore, Cr(VI) production in this region is independent of the chlorine content, as predicted earlier. This is evident upon comparing the Cr(VI) value produced from 0.1 ppm Cr(III) (5.0 ppm chlorine, region A, Figure 4.5) with the Cr(VI) value produced from the same Cr(III) increment (0.1 ppm) at less than half the chlorine content (2.0 ppm chlorine, region A, Figure 4.4). The Cr(VI) values in region A (0.1 ppm Cr(III) added) are almost identical in spite of the large variation in chlorine level occurring between Figures 4.4 and 4.5.

At first glance, Figure 4.5 would seem to deviate from the model to some extent in region A and at the first equivalence point B where the absolute concentrations of Cr(VI) appear to be somewhat lower than the stoichiometric conversions predicted by the model. (Assuming 100% Cr(III) oxidation in these regions of the

titration, the model would have predicted Cr(VI) values exactly equalling the starting Cr(III) values). These deviations can however be simply explained by the finite equilibrium constant for Cr(III) oxidation and the relatively dilute nature of the entire titration.

The data of Figure 4.1 and the excellent agreement of Figure 4.5 with the model of Figure 4.2 collectively lead us to believe in the two step oxidation mechanism proposed for low levels of Cr(III) lignosulfonate, and to believe in the protective role of the lignosulfonate moiety in preventing Cr(III) oxidation at high concentrations of Cr(III) lignosulfonate. This holds for all levels of chlorine studied here and for any level of chlorine possibly occurring in tap water.

Short Term Reactions and Temporal Variation.

Appendices B, C, and D illustrate the time dependent behavior of Cr(III) lignosulfonate oxidation by chlorinated tap water. For these experiments, the Cr(VI) was measured daily at first, and then weekly as the reactions slowed down. Appendices B and C contain time resolved data for the two Cr(III) concentrations found to be significant earlier in Figure 4.1 (Appendix B is for 0.1ppm Cr(III) and Appendix C is for 0.2 ppm Cr(III)). Contained in those two appendices are families of Cr(VI)

production (over time) at a variety of chlorine levels. (The Cr(III) is constant within an appendix, and both the chlorine level and reaction time are varied).

Appendix D is for a constant, but deliberately elevated chlorine level of 5.0 ppm where both the Cr(III) level and reaction time are varied. Free residual chlorine data (orthotolidine method) are also superimposed over the Cr(VI) production data of appendices B - D for purposes of comparison.

A quick preliminary scan of the appendices reveals that if any significant oxidation occurs, it will almost always be completed within 4 weeks. This was the justification for refering to 4 week measurements as being "long term" or equilibrium values earlier in this chapter. More specifically, appendix B shows that chlorine reaction with 0.1 ppm Cr(III) (lignosulfonate form) occurs within 2 to 4 weeks, and appendix C shows that chlorine reaction with 0.2 ppm Cr(III) generally occurs within about 3 days.

Appendix D and Figures 4.1, 4.3 - 4.5 show that high levels of Cr(III) lignosulfonate (e.g. > 1 ppm) do not react with chlorine at any level tested at any time over a 4 week period. It would appear from these data and earlier figures in this report that the most reactive

range of Cr(III) in the lignosulfonate form is 0.1 - 0.5 ppm. At these Cr(III) levels, oxidation by normal tap water appears to be minimal (see Appendices B and C, as well as Figures 4.1, 4.3 and 4.4) as long as the chlorine level does not exceed 2.0 ppm. Since tap water is typically 0.8 - 1.2 ppm and rarely exceeds 1.5 ppm, these data show that oxidation of Cr(III) lignosulfonate by chlorinated tap water will not produce environmentally hazardous amounts of Cr(VI), regardless of how much Cr(III) lignosulfonate is present. For chlorine concentrations less than or equal to 2.0 ppm, no combination of chlorine level, chromium (III) lignosulfonate level, or time produces chromium (VI) above environmentally safe limits.

Free Residual Chlorine Data.

These data are contained throughout appendices B - D and are superimposed over the Cr(VI) production. The data basically show that chlorine is consumed by lignosulfonate whether or not Cr(III) is oxidized to produce Cr(VI). The data also show that free chlorine is consumed faster when more lignosulfonate is present (again, whether or not any Cr(VI) is produced). Additional data (not included in the appendices) with unusually large Cr(III) lignosulfonate concentrations (50

ppm) gave no significant Cr(VI) production and yet produced the fastest consumption of free chlorine. In this case, the free chlorine was removed by lignosulfonate within minutes rather than days or weeks. These observations only serve to reinforce our belief that the lignosulfonate moiety protects the Cr(III) against chlorine oxidation by preferentially eliminating the free chlorine content of the sample before it has a chance to react with Cr(III).

References

1. Standard methods for the examination of water and wastewater, XII Edition (1965)., American Public Health Association Inc, 1740 Broadway, New York, NY 10019.

2. D.Clifford and J.M.Chau., The fate of Chromium (III) in chlorinated water. (Note: Report submitted to USEPA - Not to be copied or published).

Chapter 5

General summary of the thesis results.

Conclusions.

In conclusion, it can be said that all the tasks spelled out in the introduction were completed satisfactorily. As mentioned in chapter 2, a near-direct method to determine the residual bone fragments in mechanically deboned meats was developed. The sample preparation steps consisted of shaking the mechanically deboned meats for 20 minutes in 2% nitric acid. This procedure was shown to extract calcium quantitatively from beef, chicken,turkey and pork and its accuracy was shown to be comparable to that of the homogenization method and the wet ashing method. The shaking procedure is the most convenient of the three because 48 samples may be processed simultaneously in one shaker. The new spraychamber and burner systems were shown to perform well by comparing its performance with the direct current plasma system and was shown to overcome the slightly low recovery characteristics of the old system. The new flame emission system was also shown to be free from phosphate interference under the conditions suggested. Finally, preliminary investigations showed that the method could be applied quite effectively to determine dairy additives as calcium in hotdogs.

In chapter 3, a slurry method for the determination of selenium by graphite furnace atomic absorption

spectrometry was developed. Oxygen ashing was shown to be incapable of providing conditions for efficient ashing, analyte stabilization or sufficient background reduction at 196 nm. The reduced palladium matrix modifier was shown to stabilize selenium during ashings of up to 1000 ^{o}C and to reduce the final background at 196 nm sufficiently to allow an accurate deuterium arc correction. The optimum ashing and atomization temperatures were 1000 ^{o}C and 2800 ^{o}C respectively, using the reduced palladium matrix modifier. Argon was chosen as the inert gas because it provided better sensitivity for selenium determination than nitrogen. The 196 nm line of Se was preferred over the 204 nm line since it was more sensitive and since iron interference was not a problem at the levels of iron present in the liver sample. Standard addition calibration was found to be essential in order to correct for matrix effects. The method was shown to be accurate using an NBS standard reference material.

In chapter 4 it was shown that lignosulfonate had a "stabilizing effect" to protect the Cr(III) from being oxidized by chlorinated tap water by behaving as a reducing agent towards free chlorine. It was also shown to be capable of reducing Cr(VI). The percentage

conversions of Cr(III) to Cr(VI) on both the short term and the long term (4 weeks) basis were determined for several different concentrations of chromium (III) lignosulfonate and free chlorine. A model for the titration of a known free chlorine solution with chromium (III) lignosulfonate was proposed.

In summary, several observations have been made that have opened new possibilities in slurry atomization methods and in expanding our understanding of the redox chemistries of chromium silvichemicals.

Chapter 6

Future Directions.

Future Directions.

It is our belief that the slurry method for flame emission determination of sodium, potassium and calcium using the flame photometer has now reached a very high degree of maturity. Extensive studies have been conducted in this field in our laboratory as indicated in chapter 2. The prototype of a highly automated system has been built and is currently in operation at the USDA Midwestern lab, St. Louis, MO. This consists of a laboratory computer interfaced with the flame photometer for signal and data processing, a computer controlled autosampler and a balance interface that sends sample and dilution data directly to the computer. A suggestion is being made to analysts in a variety of fields to use this system in their applications. A possible improvement would be the use of a robotic arm to dispense, weigh and cap samples, to load and activate the shaker and to transfer samples to the auto-sampling carousel of the present photometer.

The applicability of the reduced palladium modifier to selenium determination in beef liver has opened up a lot of new possibilities. The current method can be modified in many ways. The possibility of using 5% hydrogen gas mixed with argon as the reducing agent to replace the hydroxylamine hydrochloride should be

investigated. Wall atomization that is currently being used should be compared to platform atomization. This may have interesting consequences in terms of sensitivity and tube life. Our preliminary investigations show that it would be worth trying this approach for the determination of arsenic in beef liver. Mercury would be an interesting additional possibility. Analyte stabilization at high ash temperatures will be the biggest hurdle to overcome. Other matrix modifiers such as nickel, copper, magnesium and silver or a combination of the above could be investigated for analyte stabilization during the ash cycle. Other kinds of animal and plant tissue could be attempted. The eventual goal, however, is the determination of As, Se, Hg, Pb, Cd, Ni and Cr under similar analytical conditions which would pave way for their simultaneous determination by slurry atomization graphite furnace atomic absorption.

To the best of this author's knowledge, no study similar to the one reported in this thesis has been done in studying the long term oxidation of chromium (III) chloride by chlorinated tap water. Such a study should help in understanding the redox chemistry of Cr(III) species more completely. Specifically, most studies to date have been conducted on a short time frame (less than

a day) using elevated chlorine levels (greater than 2 ppm free chlorine). It is this author's opinion that long term studies conducted at normal chlorine levels (0.8 ppm to 1.5 ppm) should indicate a more realistic picture of the environmental implications of Cr(III) oxidation.

APPENDIX A

Notes About the Analytical Methods

The Orthotolidine method.

The orthotolidine method was chosen as the method to monitor chlorine in the test samples. This method was used to measure both free and combined available chlorine. The basic principle behind this method is the formation of a yellow colored complex by chlorine and orthotolidine which has a maximum absorbance at 435 nm. In this method the sample was simply treated with the orthotolidine at a pH of 1.3 or less. If the sample had a very low total organic carbon (TOC) content, the absorbance would increase rapidly, reach a maximum in less than 1 min and start to decay after that. With samples containing high total organic contents where the chlorine is in a combined state, the absorbance maxima were reached after a few minutes. Typically, maximum absorbances were obtained in 2.5 to 3.0 min at room temperature. The detection limit of this method is 10 ppb.

The Diphenylcarbazide method.

The diphenylcarbazide method was the method of choice for Cr(VI) determination. In this method, diphenylcarbazide is oxidized by Cr(VI) to diphenylcarbazone. The resulting Cr(III) is complexed with diphenylcarbazone to produce a pink colored complex

which has a maximum absorbance at 540 nm. Spectral observations were made 20 minutes after the sample was treated with the reagent. The pH of the test solutions was 6.3. The detection limit of this method was estimated to be 6 ppb.

The Leuco-Crystal Violet Method.

This new method was developed on this project as a more sensitive and potentially more selective method for Cr(VI) determination in lignosulfonate solutions. The method was unfortunately found to underestimate Cr(VI) in lignosulfonate solution due to acid-catalyzed reducing behavior of the lignosulfonate moiety. Any Cr(VI) produced was quickly reduced back to Cr(III) by the lignosulfonate whenever the solution is acidified. Unfortunately, the new leuco-crystal violet procedure for Cr(VI) involves an acidification step. It was therefore abandoned in favor of the standard diphenyl carbazide procedure which is not nearly as acidic.

APPENDIX B

Time Dependent Behavior for Oxidation of 0.1 ppm Cr(III) lignosulfonate (UNICAL).

(constant Cr(III), variable chlorine)

Figure #	Added free chlorine
1	0.5 ppm
2	1.0 ppm
3	1.5 ppm
4	2.0 ppm

○ - Cr(VI)

△ - Free Chlorine

Figure 1

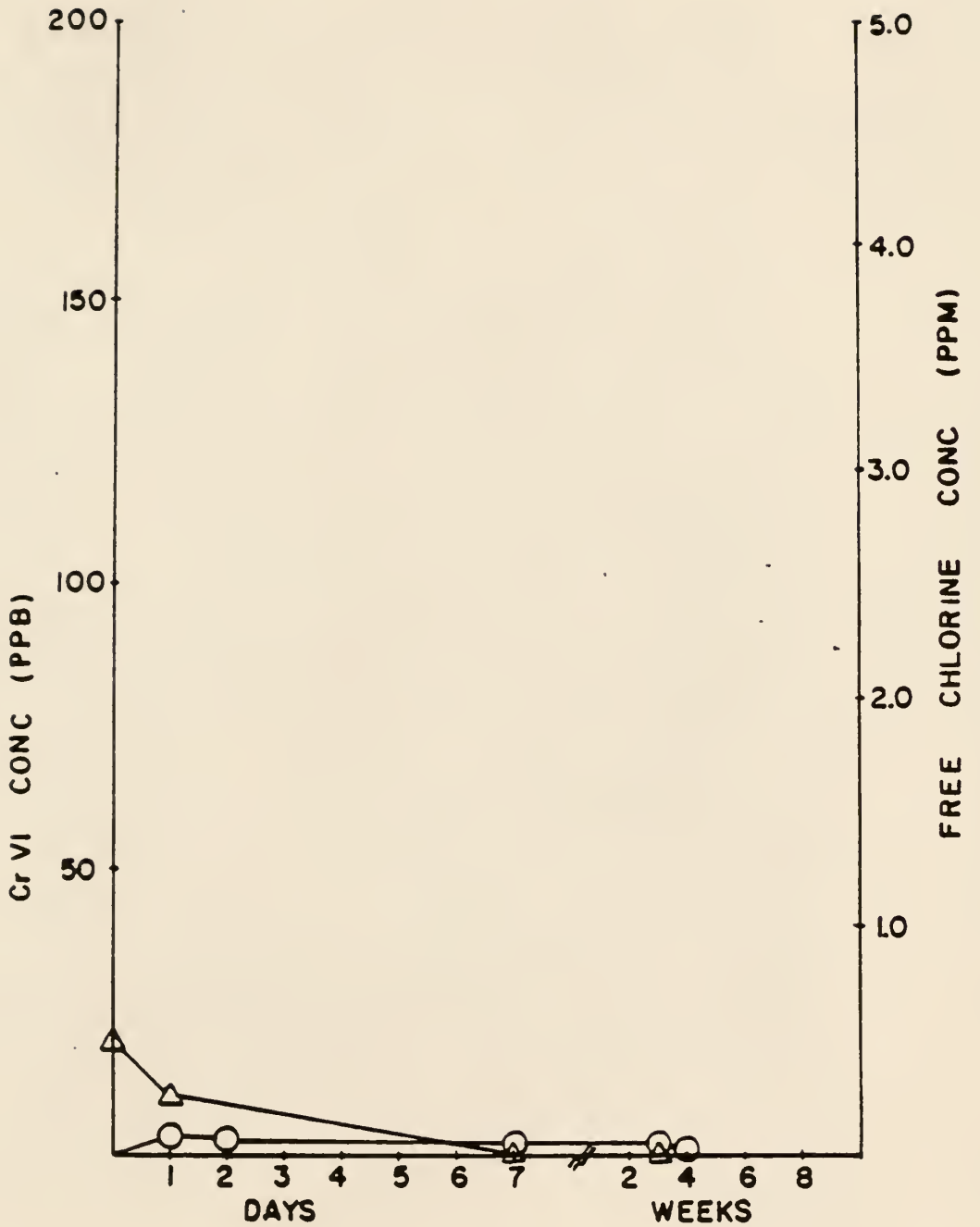

200
150
100
50
Cr VI CONC (PPB)
5.0
4.0
3.0
2.0
1.0
FREE CHLORINE CONC (PPM)
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 2

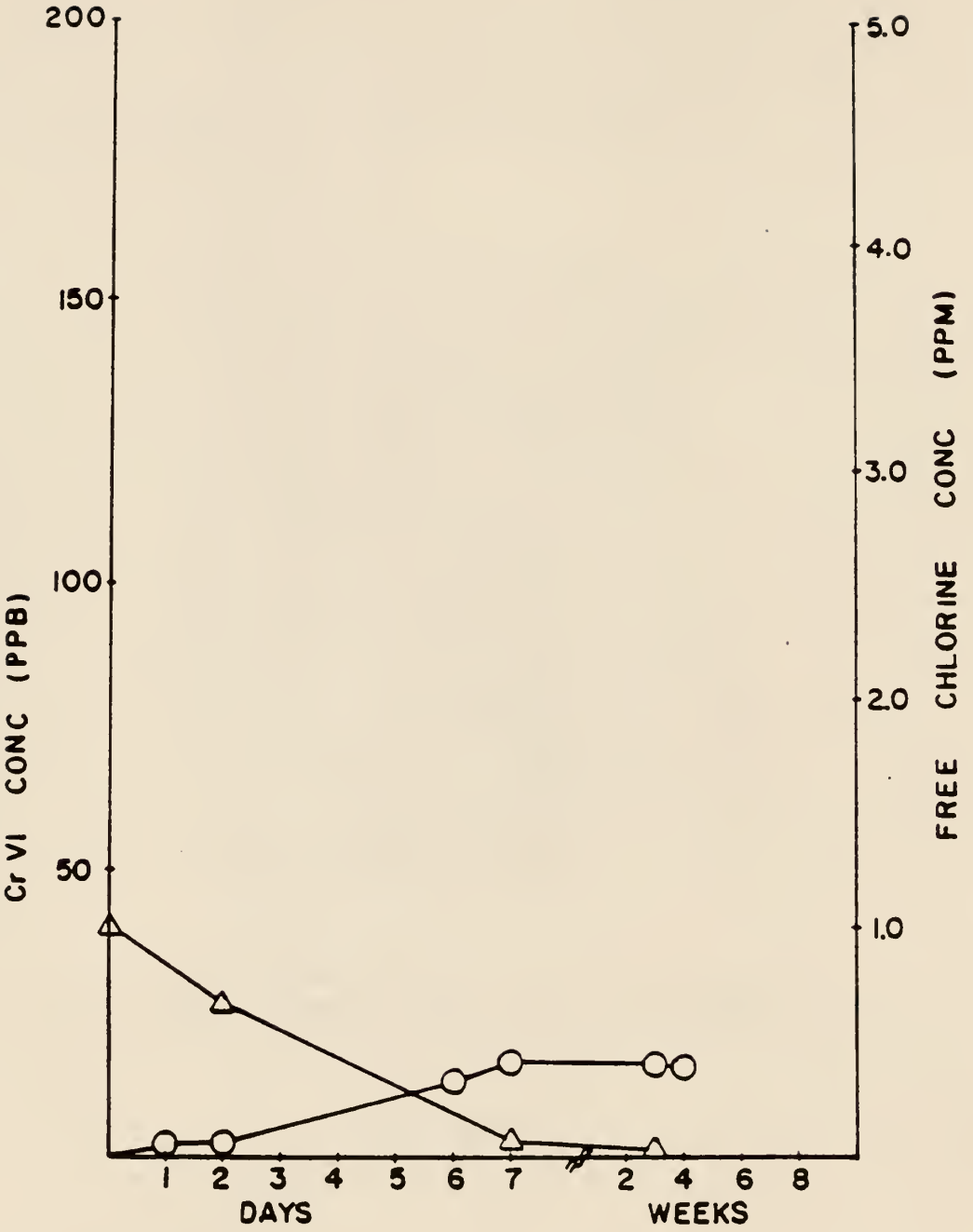
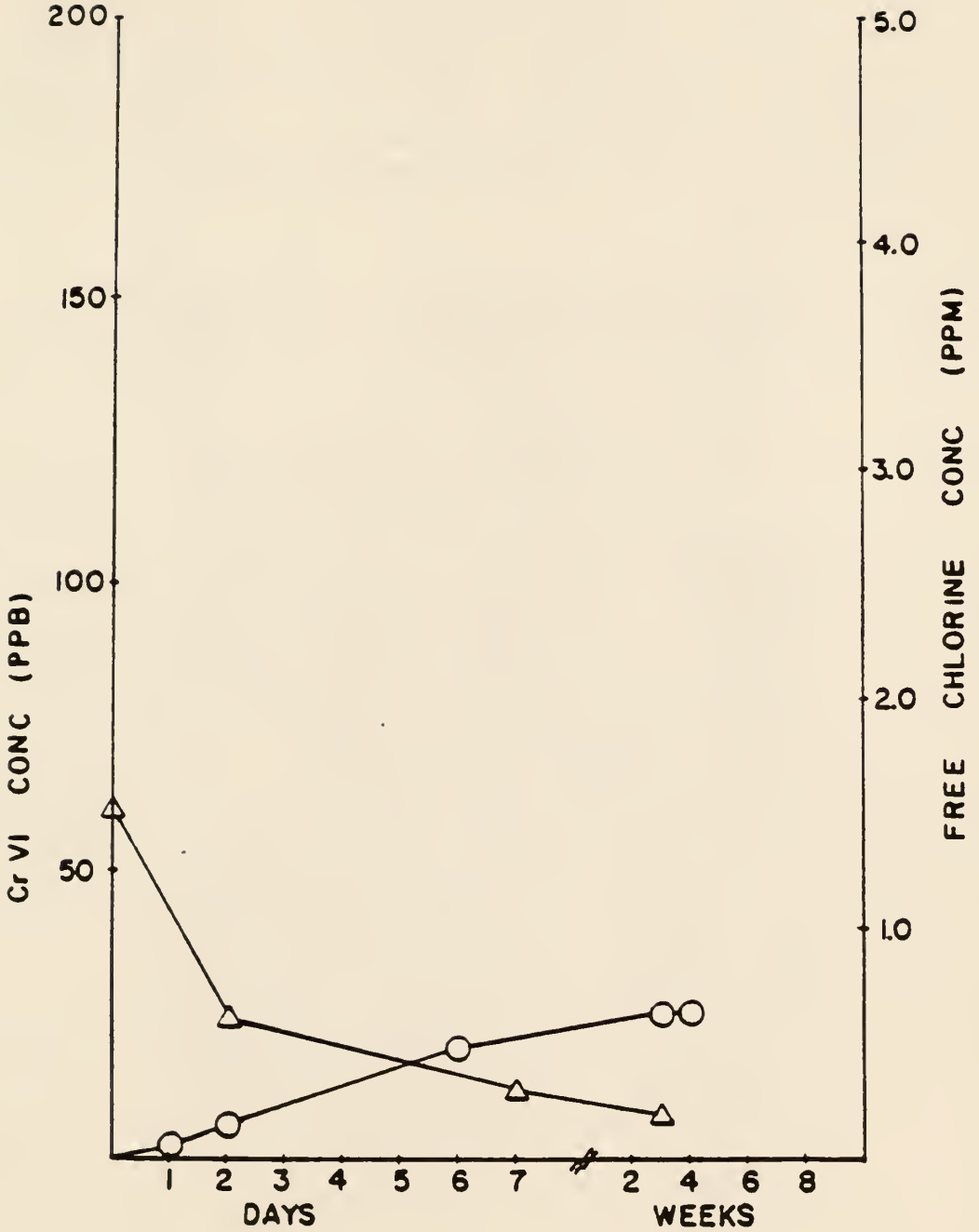

200
150
100
50
Cr VI CONC (PPB)
5.0
4.0
3.0
2.0
1.0
FREE CHLORINE CONC (PPM)
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 3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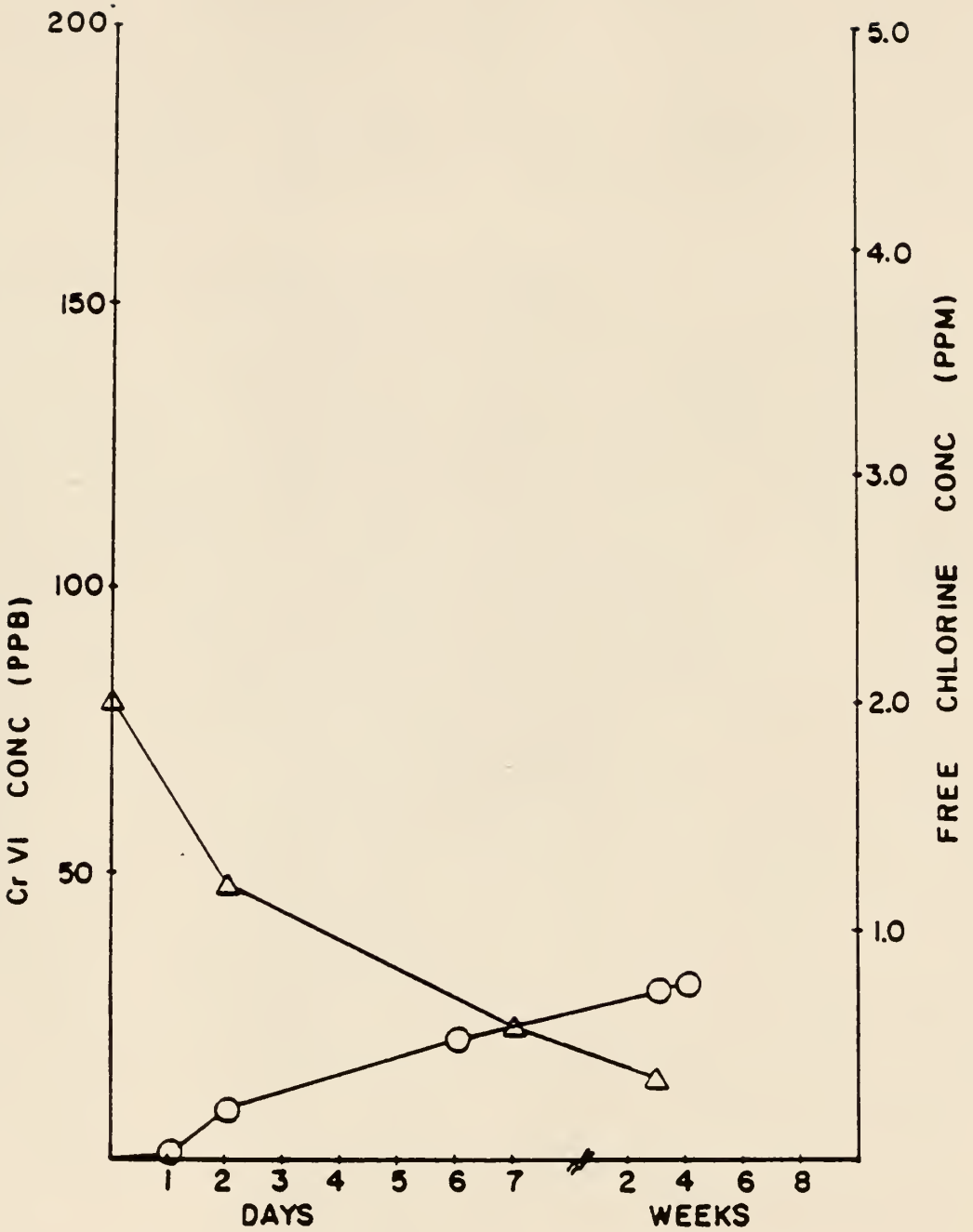
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 4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1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

APPENDIX C

Time Dependent Behavior for Oxidation of 0.2 ppm Cr(III) lignosulfonate (UNICAL).

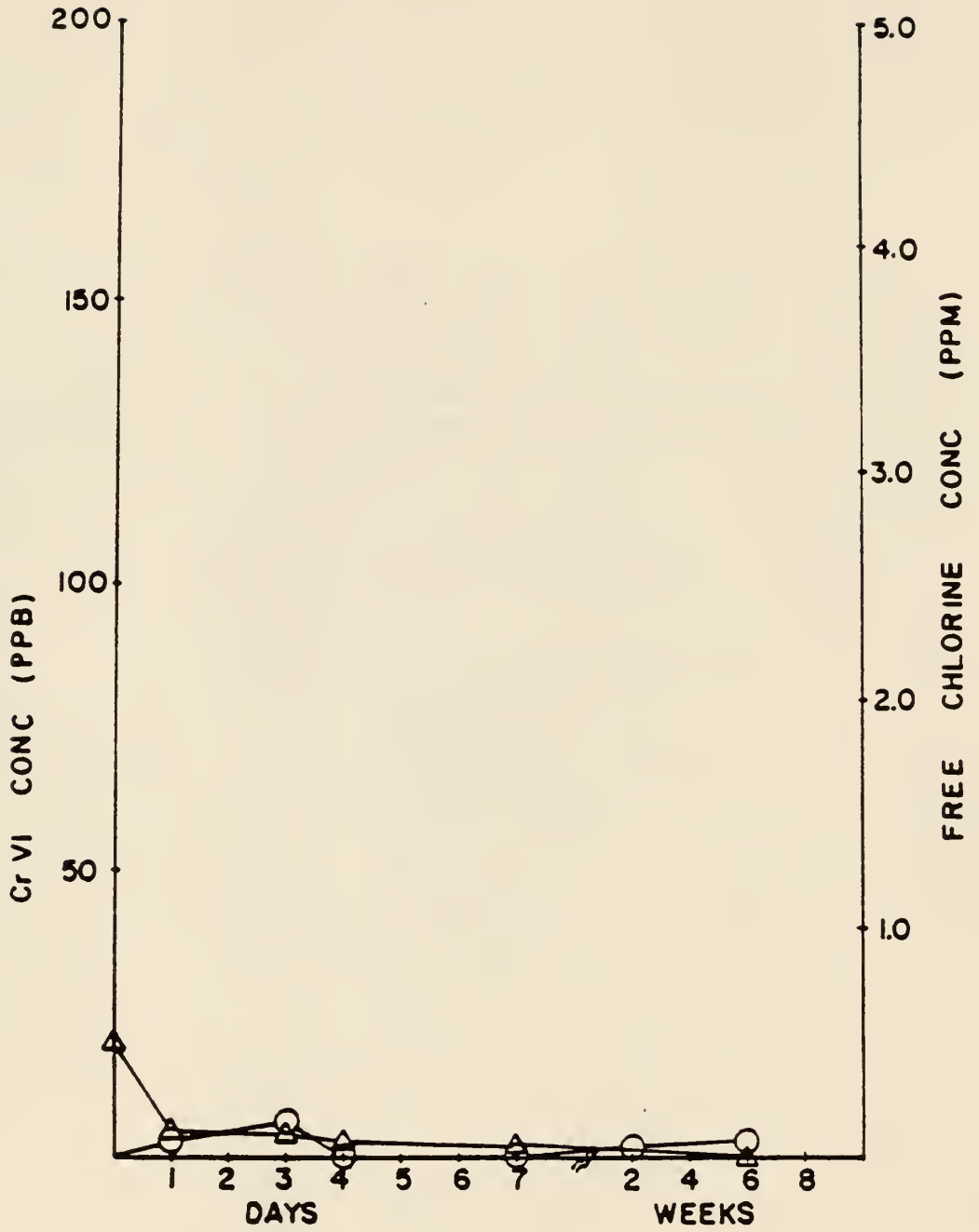
(constant Cr(III), variable chlorine)

Figure #	Added free chlorine
1	0.5 ppm
2	1.0 ppm
3	1.5 ppm
4	2.0 ppm

○ - Cr(VI)

△ - Free chlorine

Figure 1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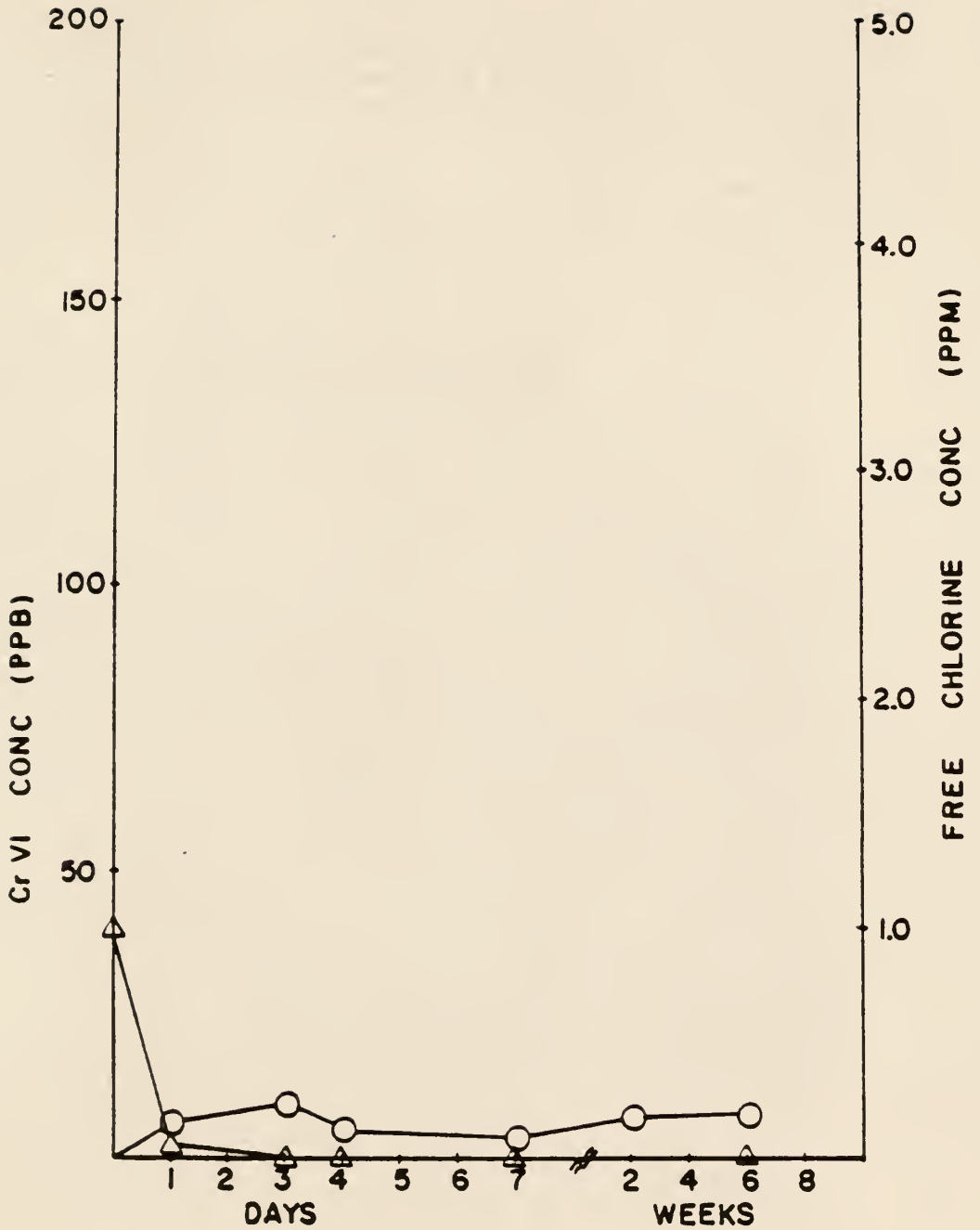
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 2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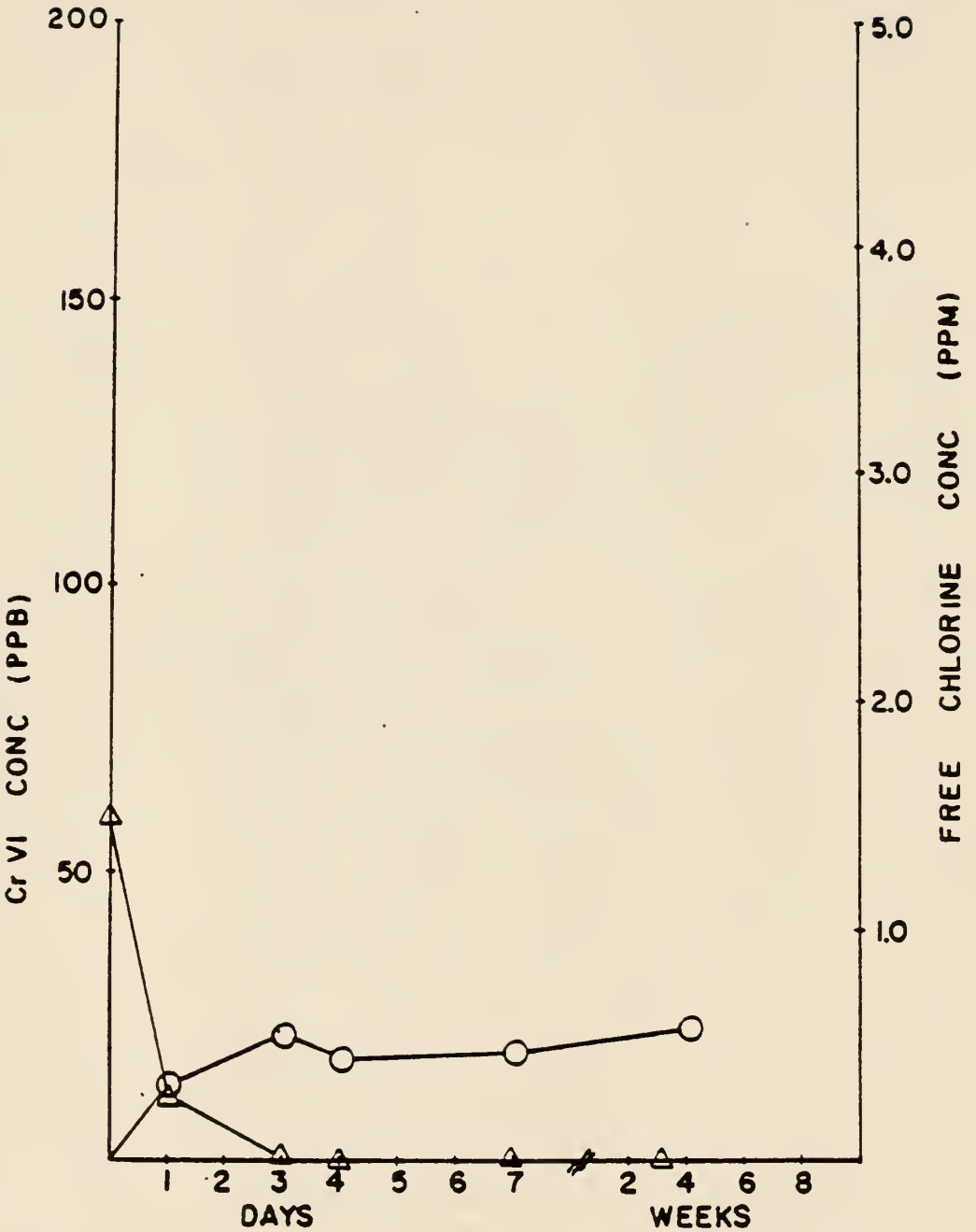
FREE CHLORINE CONC (PPM)
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 3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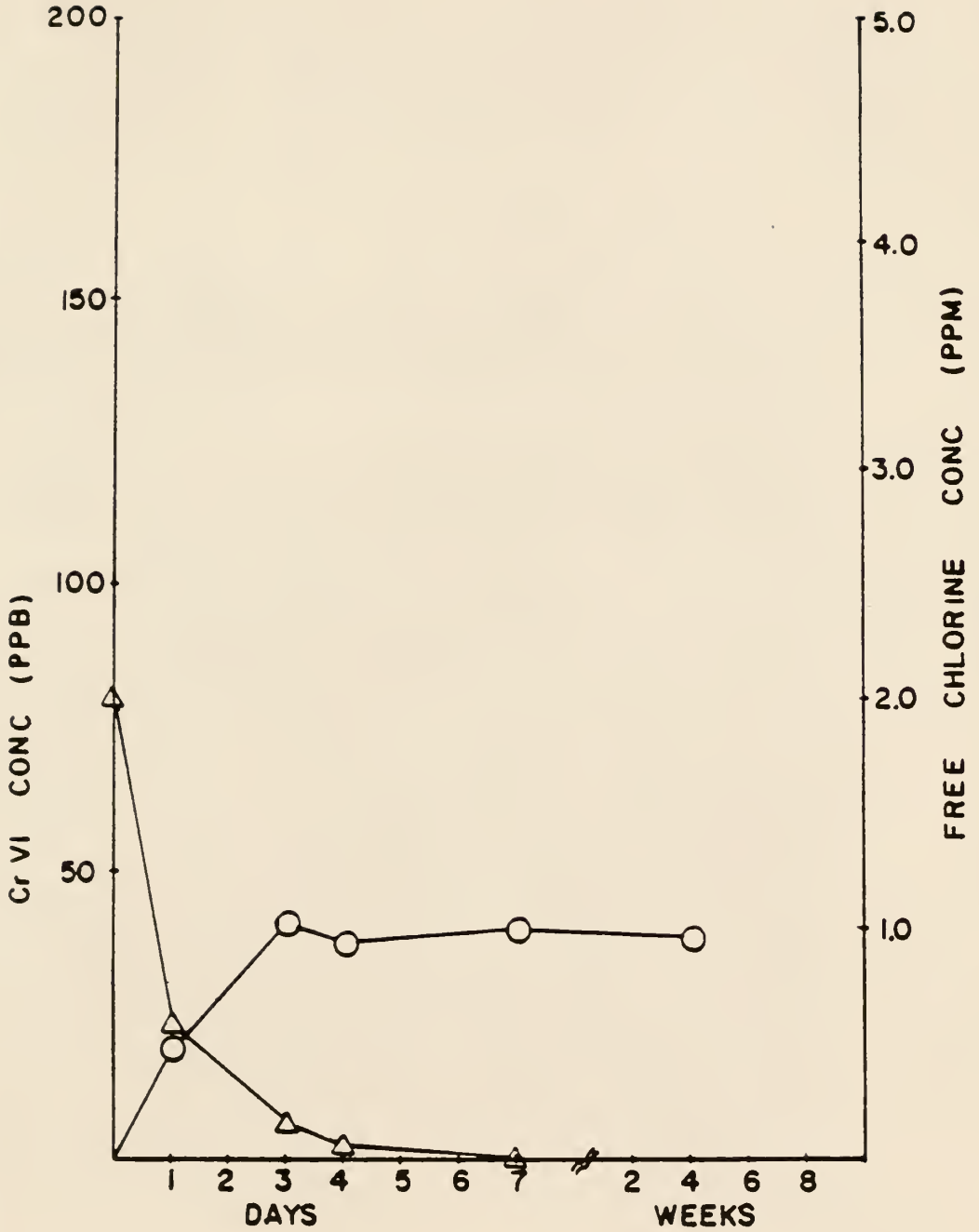
FREE CHLORINE CONC (PPM)
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 4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4.0
3.0
2.0
1.0
FREE CHLORINE CONC (PPM)
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

APPENDIX D

Time Dependent Behavior for Oxidation of Cr(III) lignosulfonate (UNICAL) by exaggerated chlorine levels (5.0 ppm)

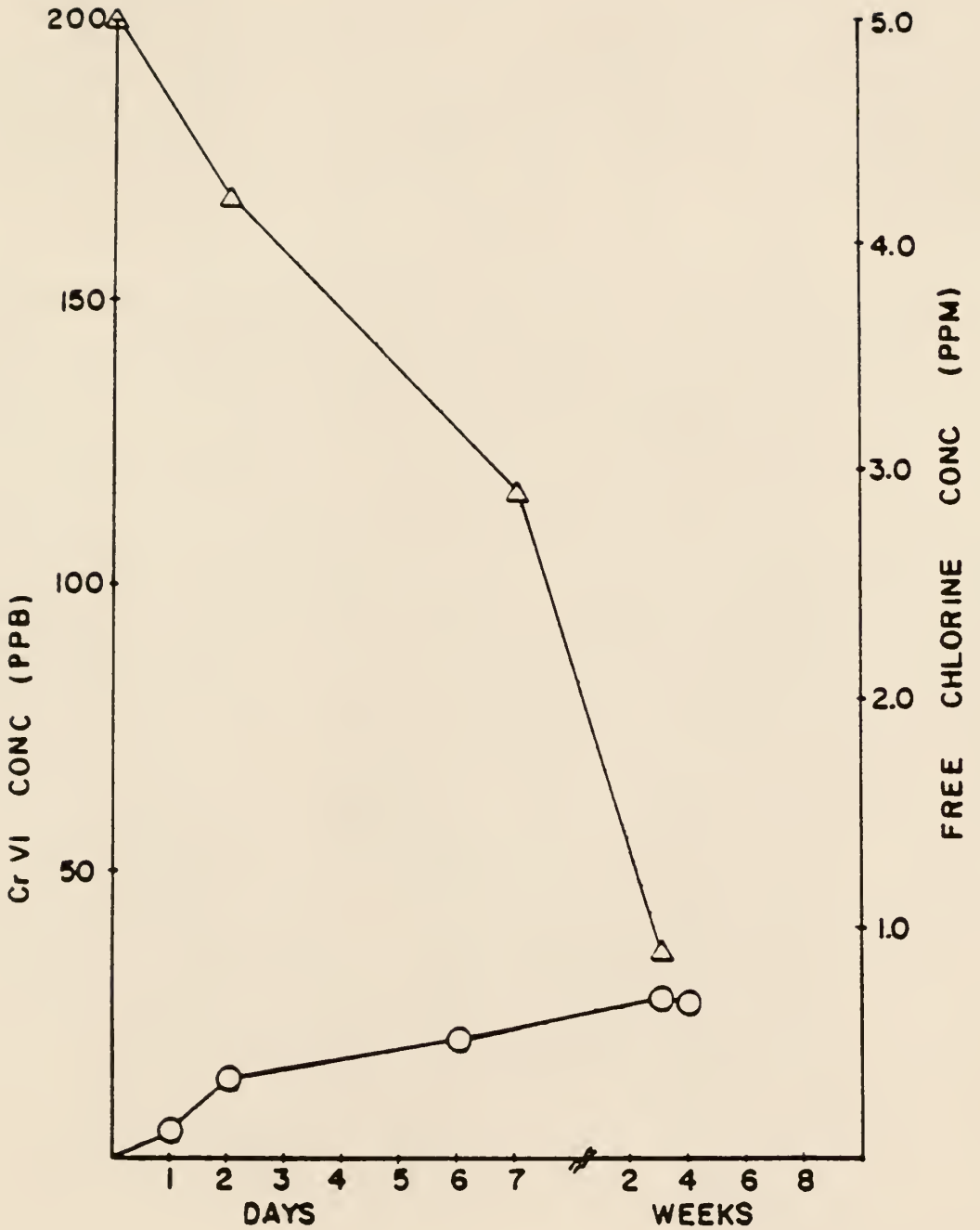
(constant chlorine, variable Cr(III))

Figure #	Initial Conc. of Cr(III) lignosusfonate
1	0.1 ppm
2	0.2 ppm
3	0.5 ppm
4	1.0 ppm
5	2.0 ppm
6	5.0 ppm
7	10.0 ppm

○ - Cr(VI)

△ - Free Chlorine

Figure 1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1.0
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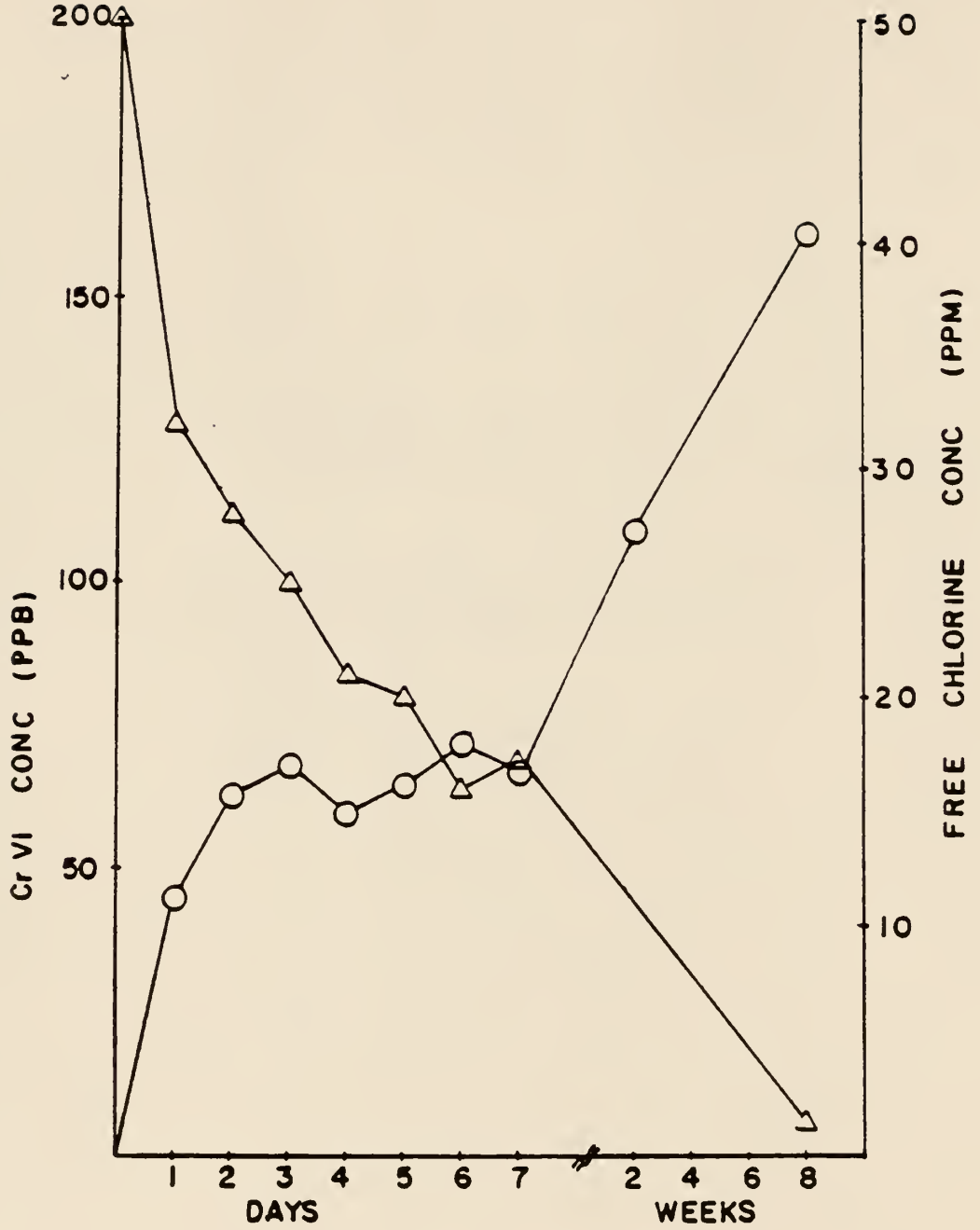
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 2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10
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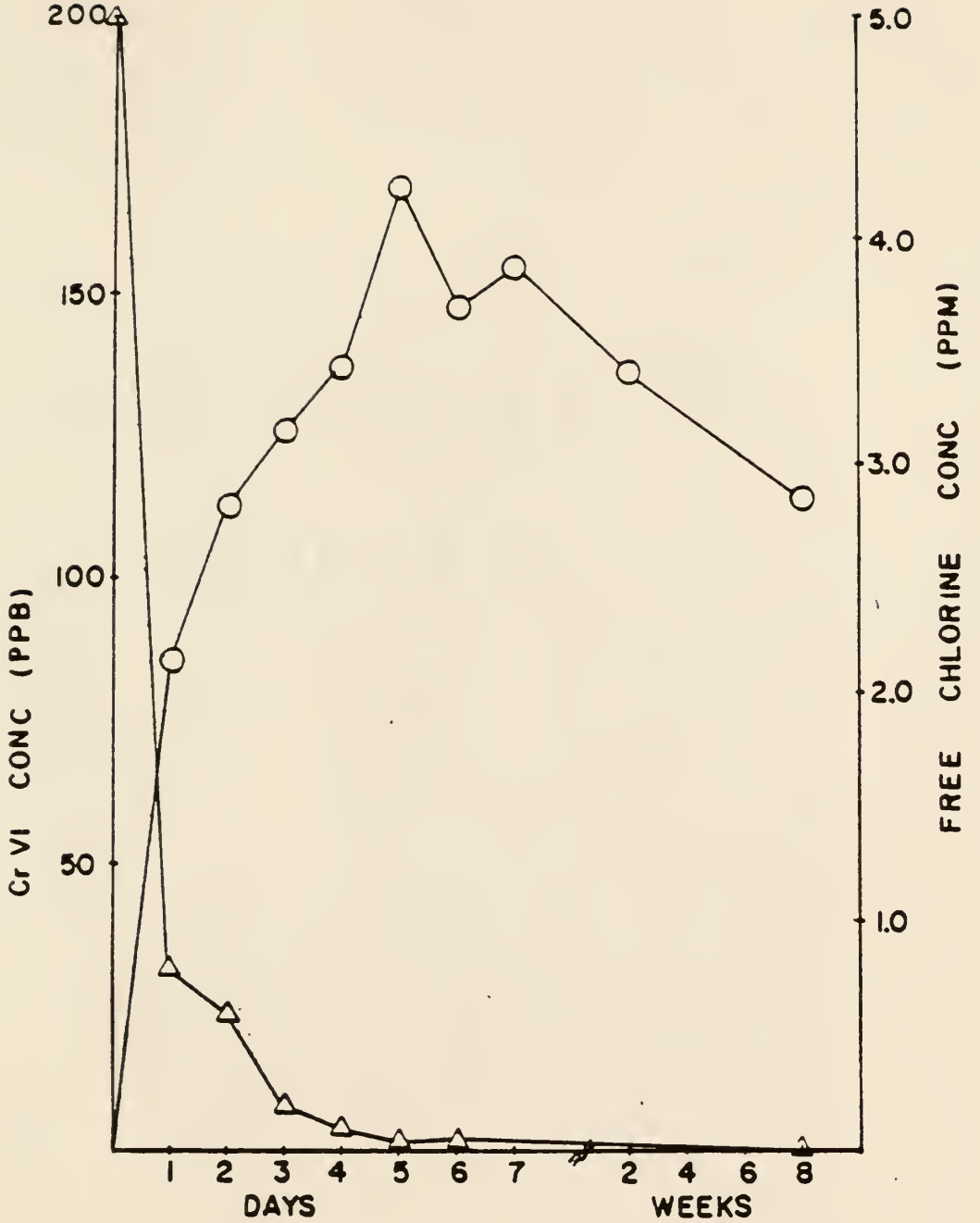
DAYS
2 4 6 8
WEEKS

Figure 3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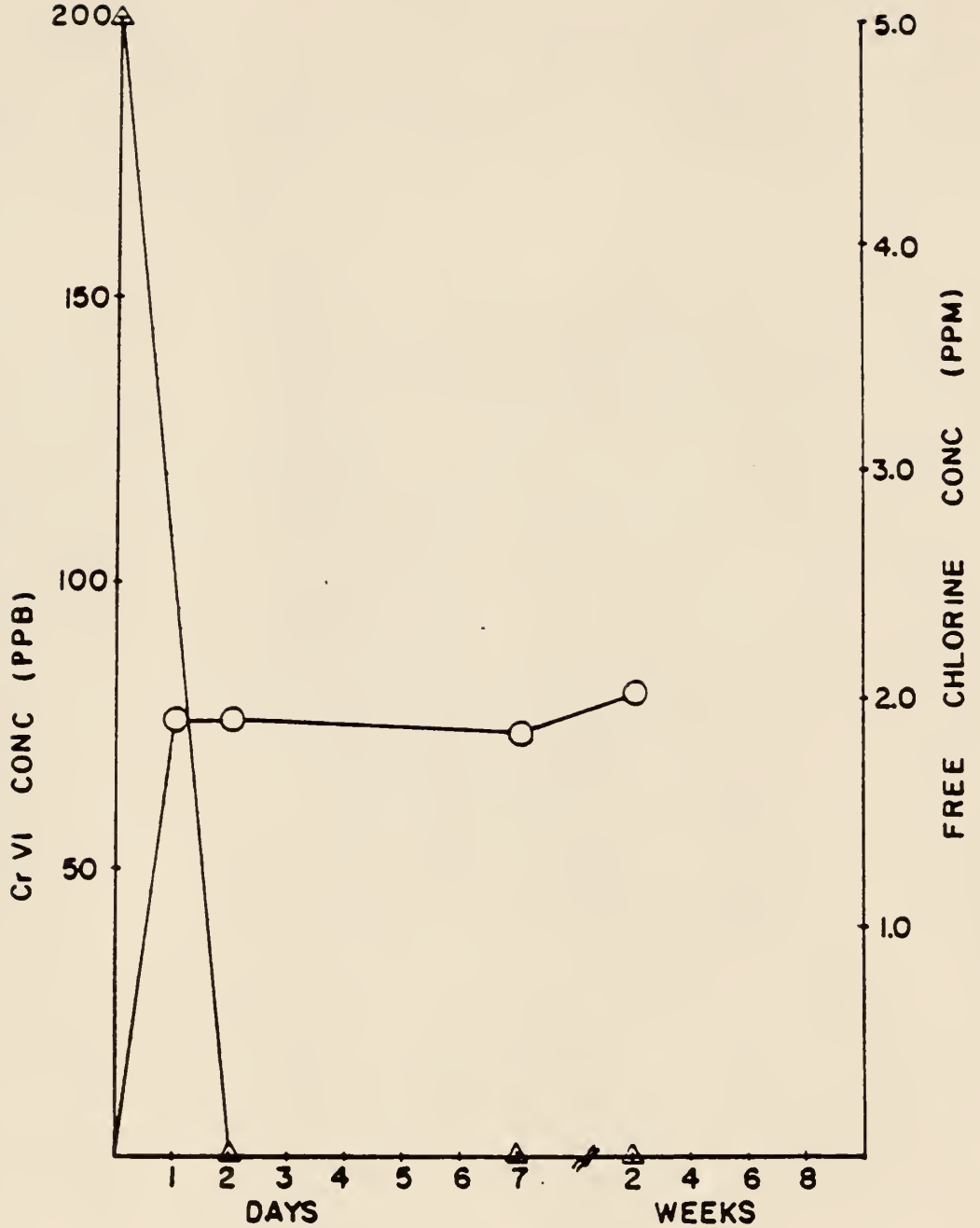
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 4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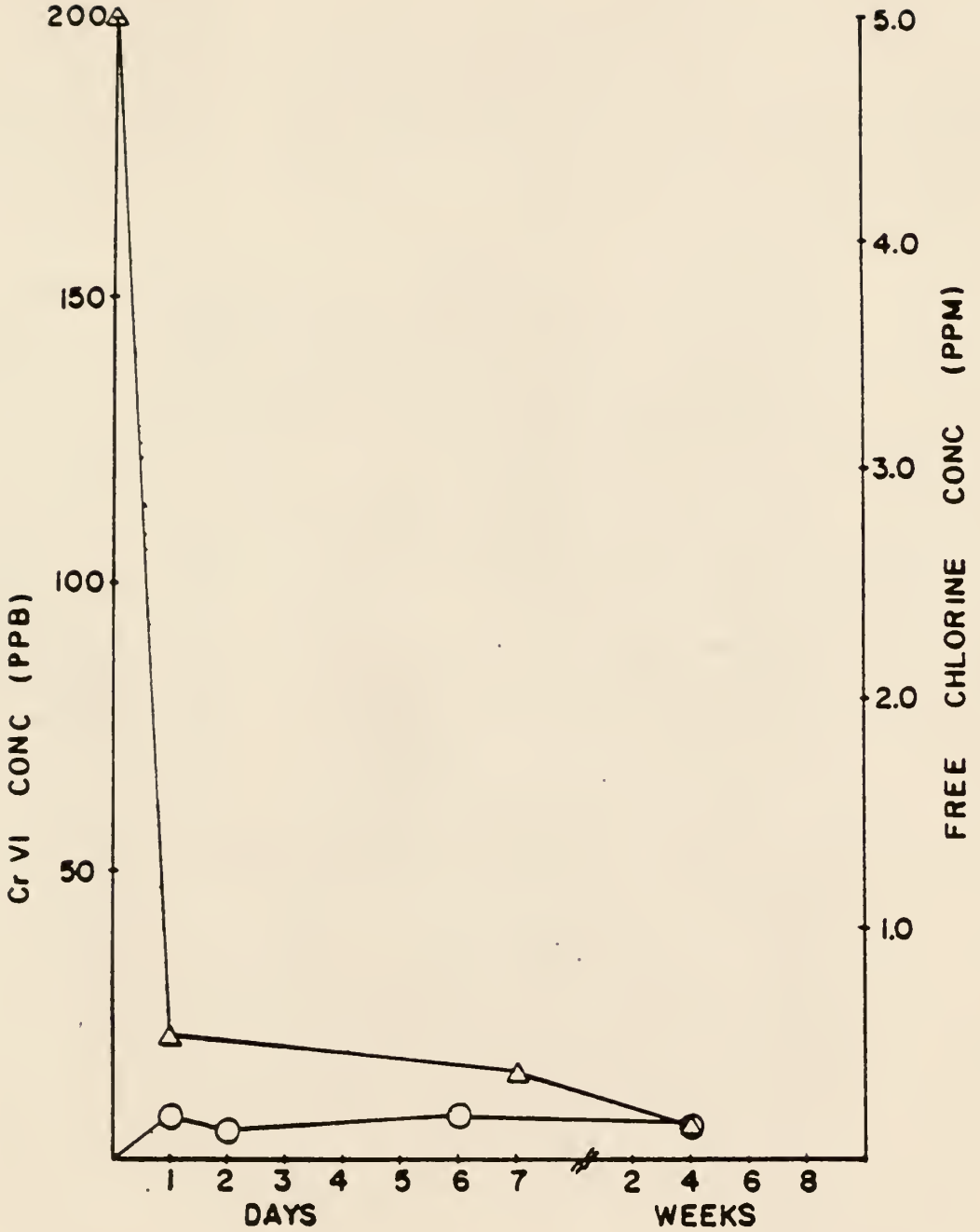
FREE CHLORINE CONC (PPM)
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 5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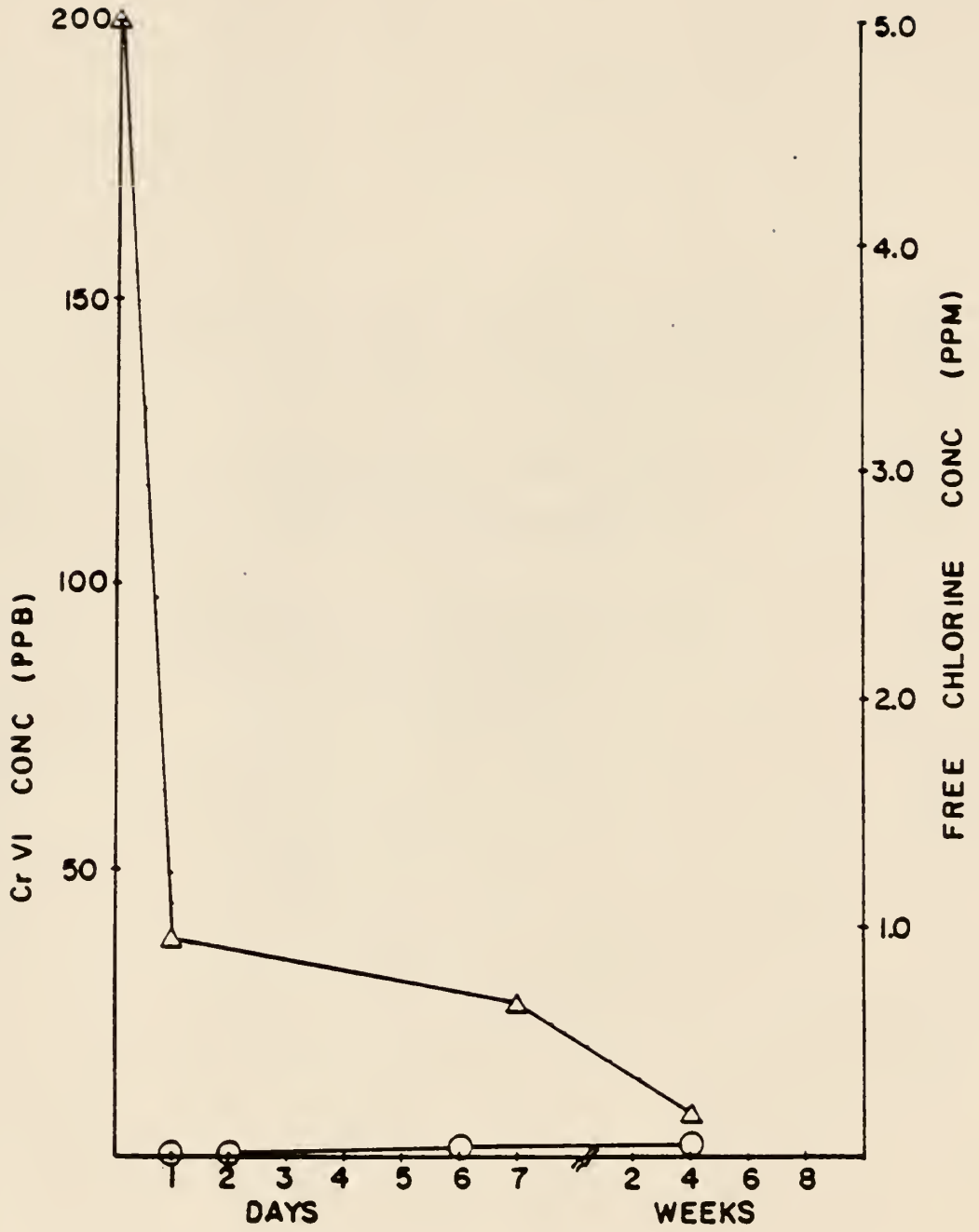
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

Figure 6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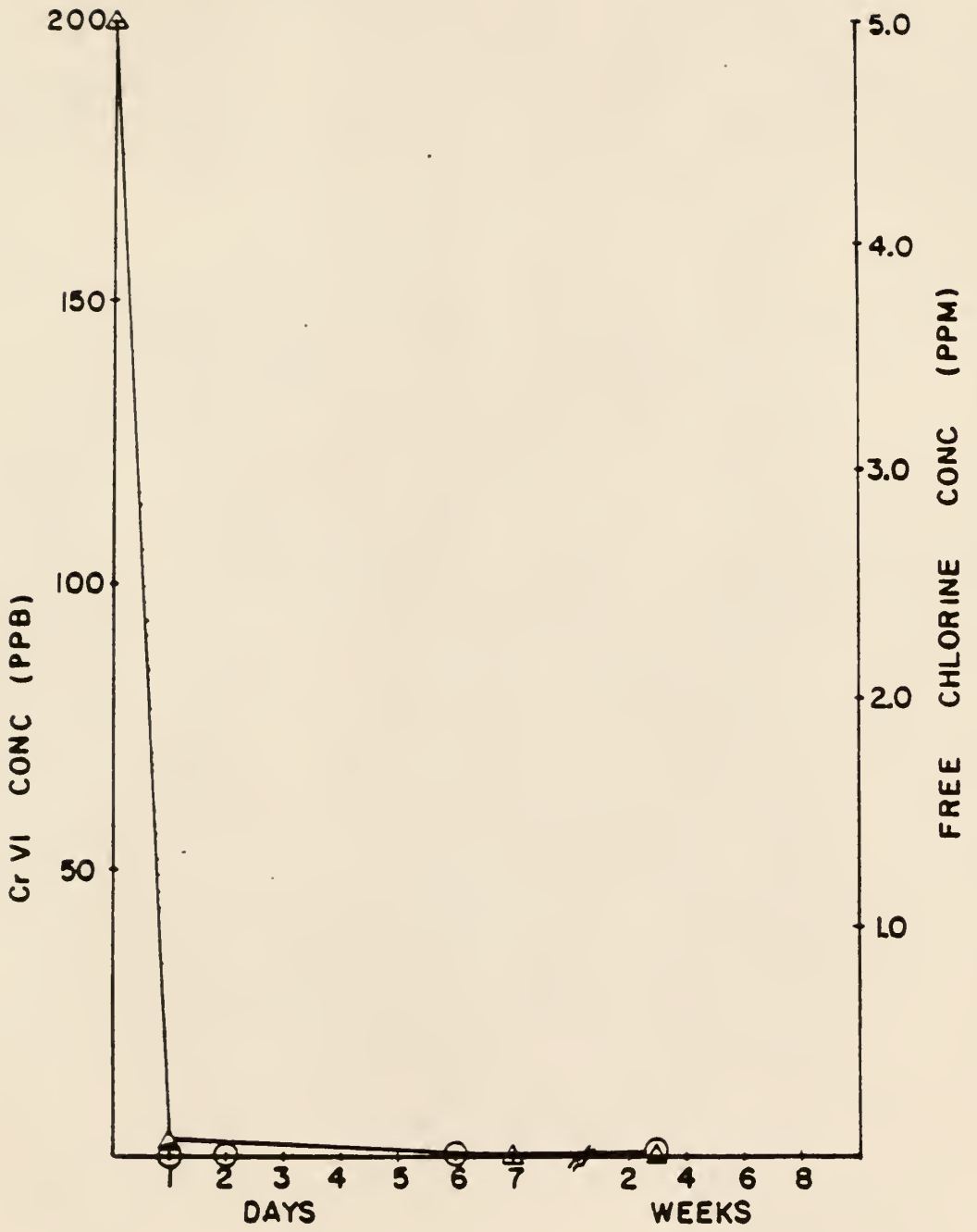
DAYS
2 4 6 8
WEEKS

Figure 7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1.0
FREE CHLORINE CONC (PPM)
1 2 3 4 5 6 7
DAYS
2 4 6 8
WEEKS

APPENDIX E

Additional data wherein the results of calcium determination by slurry atomization flame emission are compared with those obtained using the "reference" methods.

TABLE 1

Initial Group of Samples
All Data Are Original Solid Phase Calcium Concentrations (ppm)

Sample Treatment:	← Wet Ash →		← Homogenizer →	← Shaker (20 min) →				
Medium:	HCl (con.)	HNO_3 (con.)	← 2% HNO_3 at Room Temperature (dil.) →					
Method:	EDTA	Plasma Emiss.	← Slurry Atomization Flame Emission Autoanalyzer →					
			old burner →	← new burner →				
Date:			5/86	6/6	7/29	8/24	8/27	8/28
Analyst*:		Miller	Ron	Prak	Prak	Prak	Jerry,Dave	Jerry,Dave
Analyzed at:	USDA	CESL	St. Louis	KSU	KSU	KSU	St. Louis	St. Louis
Column number:	1	2	3	4	5	6	7	8
I.D.# Type								
19632 chicken	800	820	720	1000	780	790	790	
19645 chicken	900	990	890	1100		850	930	
19648 chicken	1200	570	480	570		570	760	770
19686 beef	500	360	310	380		350	370	
19687 beef	900	780		730		820	850	
19688 beef	1200	1100		1100		1100	1100	
19689 beef	400	450		400		400	400	
19690 beef	500	400	360	420			390	
19691 beef	1100	890		910	870	840	730	880
19752 chicken	1700	1700	1450	1600		1600	1500	
19935 pork	300	250		240	270	260	230	

Notes: *Miller = Donovan Miller, KSU Campus Emission Spectroscopy Lab.
Ron = Ronald Fietkau, KSU Chemistry (now at Texas A&M).
Prak = Prakash Venkatesan, KSU Chemistry.
Jerry,Dave = Jerry Montgomery and Dave Milner, USDA Midwestern Lab, ST. Louis (results they obtained using the new slurry atomization flame emission autoanalyzer).

TABLE 3

SECOND MAJOR SAMPLE GROUP
(All data given as weight % Calcium)

Sample Treatment:	←——— Wet Ash ———→		Shake 20 min.
Medium:	HCl (conc.)	HNO_3 (hot conc.)	2% HNO_3 (dil.) room temp.
Method:	EDTA Titration	Plasma Emission	Slurry Atomiz. Flame Emission
Date:		9/86	8/28/86
Analyst:	USDA	Prakash	Jerry, Dave
Analyzed at:	USDA St. Louis	CESL, KSU Manhattan	USDA St. Louis
Column number:	1	2	3
I.D.# Type			
27986 chicken, FG	0.073	0.097	0.096
29523 ?	0.036	0.041	0.042
29564 chicken, MSM, Y	0.201	0.19	0.17
29713 turkey, C, Y	0.159	0.17	0.12
29864 chicken, FG	0.128	0.14	0.13
29935 chicken	0.260	0.26	0.18
29992 chicken, Y	0.151	0.15	0.15
30006 chicken, MSM, P	0.044	0.037	0.037
30797 chicken, FG	0.097	0.092	0.12
30911 chicken, FG, BR	0.094	0.075	0.070
30919 chicken, MSM, P	0.051	0.048	0.047
31153 chicken, M, C	0.207	0.16	0.12
31230 chicken, FG	0.187	0.15	0.14
31232 chicken, FG	0.204	0.17	0.18
31233 chicken, FG	0.187	0.18	0.16
31234 chicken, FG	0.170	0.18	0.15

Key: FG = finely ground; MSM = mechanically separated meat; Y = young; C = cooked; P = parts; BR = breast and rib; M = mature

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First and foremost I would like to express my most sincere appreciation and gratitude to my advisor Dr. Robert C. Fry, not only for his guidance and help throughout my graduate program, but also for being a warm host throughout my stay in the United States. I would like to thank Dr. Kenneth Klabunde and Dr. Clifton Meloan for serving in my committee and assisting me in my program. I would also like to thank Drs. Bill Fateley, Dale Hawley, Eric Maata, Keith Purcell and all the other faculty, equally important, for their help and encouragement.

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I would also like to express my thanks to a special friend, Arunan for being understanding and thoughtful like only he can.

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VITA

Prakash Venkatesan was born on september 8, 1965. His elementary, junior and high school years were spent in the towns of Tirunelveli, Dindigul and Madras respectively, in the state of Tamil Nadu in India. He graduated from high school in 1982 and in the same year proceeded on to the University of Madras to pursue his studies toward the Bachelor of Science (B.Sc.) degree in Chemistry. He obtained the B.Sc. (Chemistry) degree in june 1985 and arrived at Kansas State University in fall 1985 to work toward the Master of Science (M.S.) degree in Chemistry. He completed the requirements for the M.S. degree (Analytical Chemistry) in spring 1988.

SELECTED APPLICATIONS OF ANALYTICAL SPECTROSCOPY TO FOOD AND ENVIRONMENTAL CHEMISTRY

by

PRAKASH VENKATESAN

B.Sc., University of Madras, 1985

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1988

Abstract

Investigations were conducted in order to develop slurry methods for the rapid determination of residual bone fragments (as calcium) in mechanically deboned meat products by flame photometry and selenium in beef liver by graphite furnace atomic absorption spectrometry. Studies were also undertaken to gain insights into the redox chemistry of Chromium (III) lignosulfonate in the presence of chlorinated tap water.

Mechanically deboned meat samples were simply shaken with 2% nitric acid for 20 minutes using a mechanical shaker and sprayed directly into an air-acetylene flame. Calcium was solubilized from the bone fragments, but the protein and fat bulk of the sample was largely unaltered. Quantitative calcium recoveries were obtained in a slurry approach as compared to wet ashed samples of the same meat. Phosphate interference in Ca determination was overcome by using a spray chamber insert to reduce the liquid aerosol droplet size for solubilized calcium. The method was shown to be applicable to beef, chicken, pork and turkey.

In order to determine selenium in beef liver, a 10% beef liver slurry was prepared by homogenizing the liver tissue along with water for 2 minutes using a Brinkmann Polytron homogenizer. This was then placed in a

pyrolytically coated graphite tube along with the palladium-hydroxylamine hydrochloride matrix modifier, ashed at 1000 °C and atomized at 2800 °C. Atomic absorption results with this system agreed well with those obtained with wet ashed samples and the entire analysis was indirectly validated by determining an NBS standard reference material.

The oxidation of chromium (III) lignosulfonate by chlorinated tap water was studied by reacting several different concentrations of chromium (III) lignosulfonate with various concentrations of hypochlorite solutions. At normal chlorination levels in tap water, lignosulfonate was found to prevent Cr(III) oxidation by reducing the free chlorine. However at elevated free chlorine levels, some Cr(III) was found to be oxidized to Cr(VI). A model for the reaction was proposed.